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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

ISSN 0706-652X / CJFSDX 52(Suppl. 1) 1–253 (1995)

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Volume 52, Supplement 1, 1995

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 52, supplément 1, 1995

Journal canadien des sciences halieutiques et aquatiques

Publié par le Conseil national de recherches du Canada

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Parasites of aquatic organisms: a Festschrift dedicated to Dr. Leo Margolis, O.C., Ph.D., F.R.S.C.

Les parasites des organismes aquatiques : un numéro spécial en l'honneur de M. Leo Margolis, O.C., Ph.D., F.R.S.C.

August 1995

Sponsored by the Department of Fisheries and Oceans, Canada

août 1995

Parrainé par le ministère des Pêches et des Océans, Canada

Special Editor/Directeur scientifique spécial

J. Richard Arthur, Ph.D., Institut Maurice-Lamontagne, Mont-Joli, Québec

Citation for complete issue/Citation complète

Arthur, J.R. [ed.] 1995. Parasites of aquatic organisms: a Festschrift dedicated to Dr. Leo Margolis, O.C., Ph.D., F.R.S.C. Can. J. Fish. Aquat. Sci. 52 (Suppl. 1): 253 pp.

Special Editor's Remarks

It is a great pleasure, to present, on behalf of the international community of ichthyoparasitologists, this Festschrift to Dr. Leo Margolis, our friend, esteemed colleague, and mentor.

Contributors of the 32 articles contained herein were chosen both for their parasitological expertise and for their association with Leo Margolis. Many have had the privilege of having worked with Leo in the Parasitology Laboratory at the Pacific Biological Station, either as fellow employees of the Department of Fisheries and Oceans (DFO), or as visiting researchers, post-doctoral fellows, or graduate students. They originate from some 15 countries (Australia, Canada, the Czech Republic, Denmark, Finland, France, Japan, Mexico, Norway, the Philippines, Russia, the Ukraine, Tunisia, the United Kingdom, and the United States), testimony to the fact that Leo and his work are known and admired around the parasitological world. Included in this volume are descriptions of five new species of fish parasites (3 Digenea, 1 Nematoda, and 1 Copepoda) named in his honour.

The preparation of this special volume was made easier by the active support of a large number of colleagues. In particular, Norbert P. J. Boyce, Z. "Bob" Kabata, and the staff of the *Canadian Journal of Fisheries and Aquatic Sciences* provided invaluable guidance and encouragement throughout the planning and editing process. Additional thanks are also due to Dr. Kabata for enthusiastically agreeing to write the dedication to this volume. Likewise, the whole-hearted support of members of the DFO's Atlantic Parasitologists Working Group was essential to its success.

I also gratefully acknowledge Jean Boulva, Quebec Region Science Director, and Serge Labonté, Research Manager, Fish and Marine Science Division, Maurice Lamontagne Institute, for allowing me to undertake this project.

Financial support for publication was provided by contributions from DFO's Gulf, Newfoundland, Quebec, Pacific, and Scotia–Fundy Regions. My sincere thanks go out to Regional Science Directors John S. Loch, Larry Coady, Jean Boulva, John C. Davis, and James A. Elliott for making this work possible.

Finally, I thank the authors, both published and unpublished, who submitted manuscripts for this special volume, and the more than 80 colleagues who acted as reviewers for these articles.

Mot du directeur scientifique

Je suis particulièrement heureux d'offrir, au nom de la communauté internationale d'ichtyoparasitologues, les présents mélanges à M. Leo Margolis, notre ami, vénéré collègue et mentor.

Les soumissionnaires des 32 articles de ce numéro spécial ont été choisis autant à la lumière de leur expertise en parasitologie que de leur relation avec Leo Margolis. En effet, plusieurs de ceux-ci ont eu le privilège d'oeuvrer avec Leo au laboratoire de parasitologie de la station biologique du Pacifique, soit à titre d'employés du ministère des Pêches et des Océans (MPO), de chercheurs invités, de boursiers postdoctoraux ou d'étudiants diplômés. Ils sont issus de quelque 15 pays (Australie, Canada, République tchèque, Danemark, Finlande, France, Japon, Mexique, Norvège, Philippines, Russie, Ukraine, Tunisie, Royaume-Uni et États-Unis), ce qui illustre le respect que la communauté internationale de la parasitologie porte à Leo et à ses travaux. On trouvera dans le présent Journal la description de cinq nouvelles espèces d'ichtyoparasites (3 digéniens, 1 nématode et 1 copépode), nommés en son honneur.

La préparation de ce numéro spécial a été considérablement facilitée par la participation d'un grand nombre de collègues. En particulier, Norbert P. J. Boyce, Z. « Bob » Kabata et le personnel du *Journal canadien des sciences halieutiques et aquatiques* se sont avérés généreux en aide et en encouragement tout au long du processus de planification et de rédaction. Je ne saurais oublier de souligner l'enthousiasme de M. Kabata à l'idée de rédiger la dédicace du présent numéro. Je désire aussi exprimer ma reconnaissance aux membres du groupe de travail en parasitologie de l'Atlantique du MPO, dont l'appui sans borne a permis de réaliser la présente publication.

Mes sincères remerciements aussi à Jean Boulva, directeur scientifique de la région du Québec, et à Serge Labonté, gestionnaire de la recherche, division science des poissons et des mammifères marins de l'Institut Maurice-Lamontagne, pour m'avoir permis d'entreprendre ce projet.

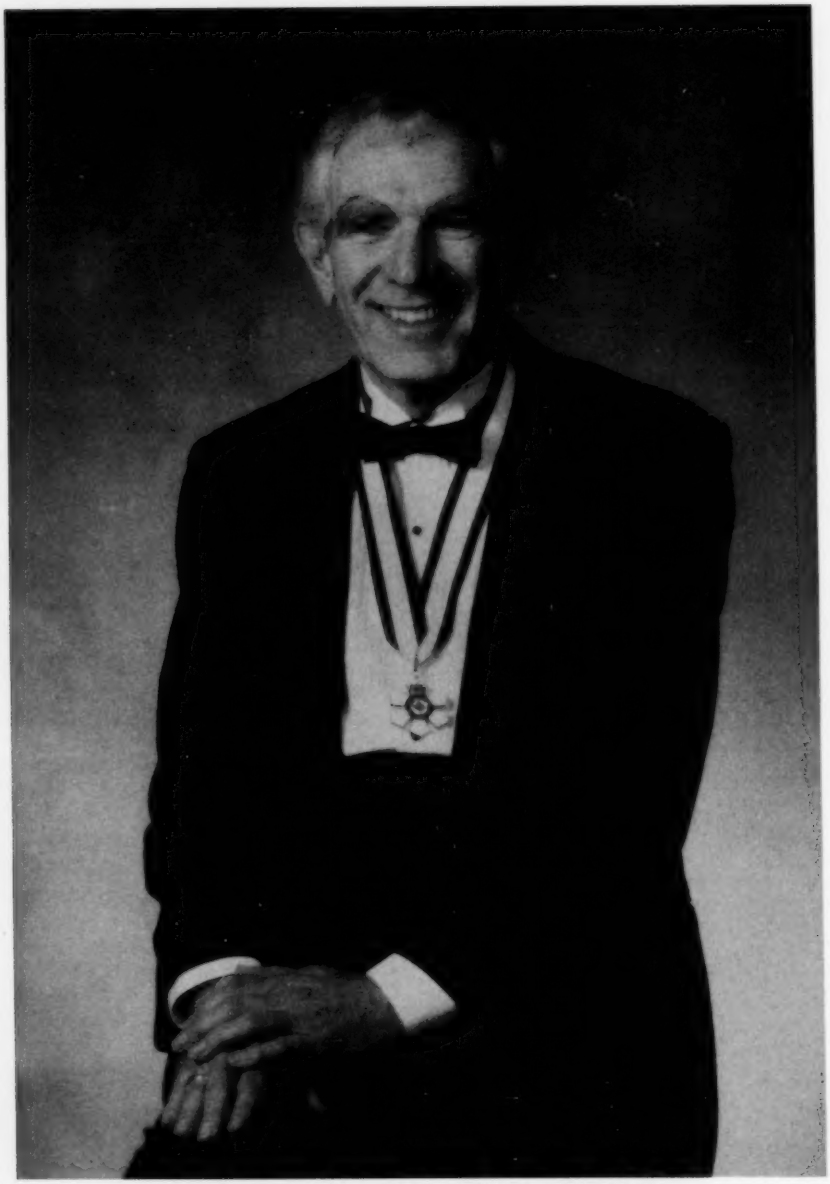
La publication de ce numéro n'aurait pas été possible sans la contribution financière des régions du Golfe, de Terre-Neuve, du Québec, du Pacifique et de Scotia–Fundy du MPO. Je remercie aussi chaleureusement les directeurs scientifiques régionaux John S. Loch, Larry Coady, Jean Boulva, John C. Davis et James A. Elliott pour avoir permis la réalisation de ce projet.

Enfin, je désire exprimer ma gratitude à tous les auteurs, que leurs travaux aient été publiés ou non, qui ont soumis des articles aux fins de publication dans le présent numéro ainsi qu'aux quelque 80 chercheurs qui ont généreusement offert de leur temps pour évaluer et réviser ces articles.

[Traduit par la Rédaction]

J. Richard Arthur

Special Volume Editor / Le directeur scientifique de ce numéro spécial



Leo Margolis, O.C., Ph.D., F.R.S.C.

Dedicated to Leo Margolis

The production of a sizable monograph in the present unfavourable circumstances calls for considerable effort. For a group of scientists to undertake, unbidden, such an effort with the aim of honouring one of their peers, implies not only that they consider him eminently worthy of such honour, but also that they hold him in affectionate esteem which makes that effort a privilege.

Leo Margolis, O.C., Ph.D., F.R.S.C. has obviously earned that honour and that esteem.

The former he earned by his outstanding scientific career. It began in Montreal, where he was born on December 18, 1927, and where he was educated at McGill University. He obtained his B.Sc. in zoology and chemistry in 1948 and his M.Sc. (Magna cum Laude) in bacteriology and immunology in 1950. He earned his spurs as a parasitologist in 1952, when he defended his doctoral thesis on parasites and diseases of fishes in the Canadian Pacific and was granted his Ph.D. by the same Alma Mater.

Apart from a short period as a Research Associate at the Institute of Parasitology, McGill University, Dr. Margolis has been associated throughout his professional life with the Pacific Biological Station in Nanaimo, British Columbia, currently an agency of Canada's Department of Fisheries and Oceans, where he joined the staff as a research scientist late in 1952 and has been a senior scientist since 1990. The first test of his skill and acumen was the task of distinguishing between North American and Asian salmon populations in the open Pacific, a problem of acute international interest at that time. A part of this now-classical investigation was a pionerring attempt at using parasites as biological tags for the purpose of fisheries management. The development and popularization of this technique, now used in many parts of the world, is largely to his credit. Its successful application by himself culminated in the first-ever case of parasite tags being used in day-by-day regulation of a valuable mixed-stock salmon fishery, a feat yet to be emulated.

However, his interests have been much broader. They have embraced the parasites and diseases of cultured and wild freshwater and marine fishes and their control, the population biology and ecology of parasites of aquatic animals, parasite transfaunation and introduction through river diversions, the economic importance of fish parasites and their significance to public health, and the sea life of Pacific salmonids.

Those interests have found an admirable expression in numerous (ca. 150) scientific papers, published in many journals and countries. Many of them do, and will for a long time, constitute indispensable sources of reference for all workers interested in the parasites of aquatic animals.

Dédié à Leo Margolis

Dans les circonstances actuelles, plutôt défavorables, la production d'une monographie aussi volumineuse demande des efforts considérables. Pour qu'un groupe de scientifiques puisse entreprendre un tel effort spontanément, en vue d'honorer un de leurs pairs, il faut non seulement qu'ils le jugent tout à fait digne de cet honneur, mais également qu'ils le tiennent dans la plus haute estime, ce qui confère à leur intervention un caractère privilégié.

Leo Margolis, O.C., Ph.D., M.S.R.C., a de toute évidence mérité cet honneur et cette estime.

C'est par sa carrière scientifique exceptionnelle qu'il s'est mérité cet hommage. Elle a commencé à Montréal, où il est né le 18 décembre 1927 et où il a fait des études à l'Université McGill. Il a obtenu son B.Sc. en zoologie et chimie en 1948, puis sa M.Sc. (Magna cum Laude) en bactériologie et immunologie en 1950. Il a obtenu son Ph.D. de la même université en 1952, après avoir défendu sa thèse de doctorat traitant des parasites et des maladies de poissons dans le Pacifique canadien. C'est ainsi qu'il est officiellement devenu parasitologue.

À l'exception d'une courte période de travail à titre de chercheur associé à l'Institut de parasitologie de l'Université McGill, M. Margolis a travaillé tout au long de sa vie professionnelle pour la Station biologique du Pacifique de Nanaimo (Colombie-Britannique), organisme qui relève actuellement du ministère des Pêches et des Océans. Il y est entré à titre de chercheur à la fin de 1952 et y occupe le poste de scientifique principal depuis 1990. La première fois qu'il a mis à l'épreuve ses compétences et sa perspicacité, c'était pour établir la distinction entre les populations de saumons d'Amérique du Nord et d'Asie regroupés dans le Pacifique; il s'agissait à cette époque d'une question qui suscitait un vif intérêt à l'échelle internationale. Une partie de cette étude qui est désormais devenue classique consistait à essayer d'utiliser des parasites comme indicateurs biologiques à des fins de gestion des pêches. Le développement et la popularisation de cette technique, aujourd'hui utilisée dans de nombreuses régions du globe, lui sont largement imputables. Il en a fait une application particulièrement réussie lorsqu'il a utilisé pour la première fois des parasites comme indicateurs biologiques pour gérer au jour le jour les pêches de saumons provenant de stocks mélangés; cet exploit n'a jamais été égalé depuis.

Toutefois, ses champs d'intérêt ont été beaucoup plus étendus. Il a travaillé sur les maladies et les parasites des poissons marins et d'eau douce, en milieu naturel ou en conditions d'élevage, et sur les possibilités de les contrôler. Il s'est aussi intéressé à la biologie et à l'écologie des populations de parasites des animaux aquatiques; au transfert des parasites entre les populations animales et leur introduction dans des nouvelles régions lors des détournements de rivières; à l'importance économique des parasites des poissons et leurs effets sur la santé publique; et à la vie marine des salmonidés du Pacifique.

Il a admirablement bien décrit ses centres d'intérêt dans de nombreux articles scientifiques (environ 150) publiés dans bon

That Dr. Margolis's scientific achievements have been widely recognized is evident in the numerous invitations he has received to lecture the world over. It is impossible to to list them all here. Suffice it to say that these invitations have involved him in 30 major commitments in the course of his career. Nine universities in Canada and abroad have sought his services as an external examiner and he is currently an Adjunct Professor at Simon Fraser University, British Columbia. Among fish parasitologists anywhere his name is a "household word."

These activities are not the only ones that have claimed his time. He is an active member of seven scientific societies, often elected to their governing bodies. In 1977–1978 he served as Chairman of the Parasitology Section, Canadian Society of Zoologists, and in 1990 became the President of this Society. He has served on the editorial boards of five scientific periodicals and is at present a co-editor of two major serial publications, one dealing with parasites of Canadian fishes and the other with the biology of Pacific salmon. As an editor he is second to none in rooting out blemishes, however small, in texts that come under his scrutiny.

The regard enjoyed by Dr. Margolis has assumed various forms. Scientists in four different countries have named newly discovered parasites of fishes after him: (*Aporocotyle margolisi* (UK), *Urocleidoides margolisi* (Hungary and Canada), *Capillaria margolisi* (Czech Republic and Canada). *Acanthochondria margolisi* (Canada), and *Margolisia vidalensis* (UK). In 1975 he was elected a Fellow of the Royal Society of Canada. The Canadian Society of Zoologists awarded him the R.A. Wardle Medal in 1982, in recognition of his "outstanding contributions to the advancement of our understanding of parasitic organisms." In 1990 the Governor General appointed him to the rank of an Officer of the Order of Canada. The British Society for Parasitology made him an honorary member in 1994 and the Professional Institute of the Public Service awarded him its coveted gold medal in 1995.

Dr. Margolis's painstaking, meticulous approach to scientific matters has extended fully also to the performance of administrative duties, which he never sought but which were thrust upon him with increasing frequency, as his career progressed. He became Head of the Pacific Biological Station's Parasitology and Pathology Group in 1967 and has never again been quite free of administrative responsibilities. He acted as the Assistant Director of the Station in 1969–1970 and again in 1972. Directorial duties were entrusted to him in 1977 and 1986–1987. On several occasions he was called upon to represent Canada in trade negotiations with foreign countries, not only by his own Department of Fisheries and Oceans, but also by other Departments. His CV lists nearly 50 different committees, national and international, governmental and intergovernmental, in the activities of which he participated at various times. He served for a number of years as a scientific advisor to the Science Council

nombre de périodiques et de pays. Un grand nombre de ces articles constituent une source de référence indispensable aux personnes qui étudient les parasites des animaux aquatiques, et vont être utilisés encore longtemps.

La reconnaissance unanime des réussites scientifiques de M. Margolis est confirmée par les nombreuses invitations qu'il a reçues pour présenter des conférences à travers le monde. Il est impossible de toutes les mentionner ici. Contentons-nous de préciser que ces invitations lui ont permis de prendre 30 engagements majeurs vis-à-vis de certains organismes au cours de sa carrière. Par exemple, neuf universités du Canada et de l'étranger ont fait appel à ses services d'examinateur externe, et il occupe actuellement le poste de professeur auxiliaire à l'Université Simon Fraser, en Colombie-Britannique. Son nom est connu de tous les parasitologues spécialistes des poissons, peu importe d'où ils viennent.

Ces activités ne sont pas les seules à avoir occupé son temps. Il est membre actif de sept associations scientifiques et fait souvent partie de leurs organes dirigeants. En 1977–1978, il a occupé le poste de président de la Section de parasitologie de la Société canadienne de zoologie et, en 1990, il est devenu président de cette société. Il a siégé aux comités de rédaction de cinq périodiques scientifiques et occupe actuellement le poste de co-éditeur de deux importantes séries de publication, dont l'une traite des parasites des poissons canadiens et l'autre, des caractéristiques biologiques des saumons du Pacifique. En tant qu'éditeur, il est le mieux placé pour déceler les imperfections dans les documents qu'il examine, aussi légères soient-elles.

L'estime que s'est attirée M. Margolis s'est concrétisée de plusieurs façons. Des scientifiques de quatre pays ont donné son nom aux nouveaux parasites qu'ils ont découverts : *Aporocotyle margolisi* (Royaume-Uni), *Urocleidoides margolisi* (Hongrie et Canada), *Capillaria margolisi* (République tchèque et Canada), *Acanthochondria margolisi* (Canada) et *Margolisia vidalensis* (Royaume-Uni). En 1975, il a été élu Membre de la Société royale du Canada. En 1982, la Société canadienne de zoologie lui a décerné la médaille R.A. Wardle pour récompenser ses « précieuses contributions à l'avancement de notre compréhension des organismes parasites. » En 1990, le Gouverneur général l'a élevé au rang d'Officier de l'Ordre du Canada. La Société britannique pour la parasitologie l'a nommé membre honoraire en 1994 et il s'est mérité la très convoitée médaille d'or de l'Institut professionnel des services publiques en 1995.

Il faut ajouter que l'approche méticuleuse des questions scientifiques adoptée par M. Margolis s'est étendue aux nombreuses tâches administratives, qu'il n'a jamais réclamées, mais qu'on lui a confiées de plus en plus souvent à mesure que sa carrière progressait. En 1967, il est devenu chef du groupe de parasitologie et de pathologie de la Station biologique du Pacifique et n'a jamais, depuis lors, vraiment cessé d'assumer des responsabilités administratives. Il a occupé le poste de directeur adjoint de la Station en 1969–1970, puis de nouveau en 1972. On lui a confié un poste de direction en 1977, puis en 1986–1987. À plusieurs occasions, le ministère des Pêches et des Océans, mais aussi d'autres ministères, lui ont demandé de représenter le Canada lors de négociations avec d'autres pays. Il a siégé à plus de 50 comités différents, au niveau national ou international, gouvernemental ou intergouvernemental, et a participé à leurs activités à diverses reprises. Il a travaillé pendant un certain nombre d'années en tant que conseiller scientifique pour le Science Council of British Columbia

of British Columbia and as a consultant to the International Development Research Centre. However, his most significant involvement in this type of activity was his decades-long association with the International North Pacific Fisheries Commission, which lasted until its dissolution in 1993. He was a popular and highly respected member of the Canadian delegation. His sound, common-sense wisdom, bolstered by extensive experience, has made him a desirable participant in all kinds of professional deliberations.

All these undoubted achievements and qualities would not have, by themselves, sufficed to prompt his colleagues into honouring him with the festschrift. It was Leo Margolis the man that tilted the scales in favour of Dr. Margolis the scientist. Perhaps the best illustration of the way in which he is perceived by his colleagues and co-workers is the statement made by one of them, referring to the quality of his leadership: "Leo gives them hell and they love him for it." Indeed, as a supervisor, he has always weighed his decisions in the light of the possible effects they would have on other people. Although he has never suffered fools gladly, he has always been considerate and supportive.

In the course of my travels I found that Dr. Margolis was well known and held in high esteem far outside his immediate circle. I saw his handsomely framed photograph on the wall of an apartment in Moscow, and in Tokyo a similarly framed drawing, or rather a doodle (sorry Leo), of a trematode, signed by him. To me, these two and other facts are an indication of his "pin-up quality," very rarely possessed by scientists of my acquaintance.

His busy professional schedule does not seem to impede Dr. Margolis's involvement in extracurricular activities. It is not generally known in scientific circles that he has devoted much of his time to amateur hockey. Not only did he play it himself, when younger, but became intimately involved with the B.C. Amateur Hockey Association. He was President of this organization in 1963–1966, the youngest Canadian ever to hold such a position in any province. In recognition of his services, the Association made him an Honorary Vice-President (1968–1988), and in 1988 appointed him an Honorary Life Member. In 1989 he received its Diamond Stick Award for his services to amateur hockey in British Columbia and in 1990 the order of Merit Award from the Canadian Amateur Hockey Association.

In the course of over 35 years of service to science and to Canada, Dr. Margolis has reached a position of high distinction and respect both nationally and internationally. A good colleague, a dependable friend, and a boon companion, he richly deserves to have the esteem and affection of his peers placed on permanent record in the form of this volume of papers. I am sure that I speak not only for the contributors to the volume but for a much greater number of his fellow scientists, when I wish him many more healthy and productive years.

et à titre d'expert-conseil pour le Centre de recherches pour le développement international. Toutefois, sa contribution la plus notable à ce type d'activités s'est concrétisée par son association à la Commission internationale des pêches du Pacifique Nord, qui a duré plusieurs dizaines d'années, jusqu'à la dissolution de cette dernière, en 1993. Il était un membre populaire et très respecté de la délégation canadienne. Sa sagesse empreinte de bon sens, combinée à sa longue expérience, a fait de lui une personne dont on sollicite la participation à toutes sortes de débats professionnels.

Toutes ces réussites et qualités, que personne ne conteste, n'auraient pas à elles-seules, suffi à encourager ses collègues à lui rendre hommage de la sorte. En fait, c'est la personnalité de l'homme qui a joué en faveur du scientifique. Le meilleur exemple de la façon dont le considèrent ses homologues et ses collègues de travail est sans doute illustré par les propos tenus par l'un d'eux, qui a fait référence à ses talents en matière de leadership : « Leo leur fait la vie dure et ils lui en sont reconnaissants. » Dont acte. En tant que superviseur, il a toujours pris ses décisions en tenant compte de l'effet qu'elles pourraient avoir sur les autres. Même s'il n'a jamais vraiment supporté les gens peu sensés, il a toujours fait preuve de considération et d'un esprit d'entraide avec ses collègues.

Au cours de mes divers voyages, j'ai constaté que M. Margolis était très célèbre et tenu en haute estime par des personnes travaillant à l'extérieur de son environnement professionnel immédiat. J'ai vu une excellente photo de lui accrochée au mur d'un appartement de Moscou, et à Tokyo, le dessin, ou plutôt une tentative de dessin de trématode (désolé, Leo) signé de sa main. Il me semble que ces deux éléments, ainsi que d'autres faits, sont une preuve de sa célébrité, et c'est là une qualité que partagent bien peu de scientifiques que je connaisse.

Ses nombreux engagements professionnels ne semblent pas empêcher M. Margolis de participer à des activités parallèles. Les membres des milieux scientifiques ignorent généralement qu'il a consacré une bonne partie de sa vie au hockey amateur. Il y a non seulement joué lui-même lorsqu'il était plus jeune, mais il est également devenu membre à part entière de la B.C. Amateur Hockey Association. Il a été président de cette organisation de 1963 à 1966; il était alors le plus jeune Canadien à occuper un tel poste, toutes provinces confondues. En reconnaissance des services rendus, l'Association l'a nommé vice-président honoraire (1968-1988) et, en 1988, membre honoraire à vie. En 1989, il a reçu le Diamond Stick Award pour les services rendus au hockey amateur en Colombie-Britannique et, en 1990, l'Association canadienne de hockey amateur lui a décerné le prix de l'Ordre du mérite.

Au cours de ses 35 années passées au service de la science et du Canada, M. Margolis a imposé le plus grand respect tant au niveau national que sur la scène internationale. Collègue de travail agréable, ami fidèle et bon compagnon, il mérite tout à fait l'estime et l'affection de ses pairs, immortalisées par le présent recueil d'articles. Je suis sûr que ce ne sont pas seulement les rédacteurs de ces articles, mais également bien d'autres scientifiques, qui s'associent à moi pour lui souhaiter de pouvoir encore travailler durant de nombreuses années et demeurer en bonne santé.

Z. Kabata

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Differences in acquired and innate resistance among Pacific salmon (*Oncorhynchus* spp.) against the haemoflagellate *Cryptobia salmositica* and numbers delivered by the leech vector *Piscicola salmositica*

S.M. Bower

Abstract: Susceptible juvenile chinook salmon (*Oncorhynchus tshawytscha*) were protected from otherwise lethal challenges with the haemoflagellate *Cryptobia salmositica* by acclimation to an elevated water temperature (20°C). Following challenge at temperatures advantageous to the haemoflagellate (9–11°C), surviving fish had plasma with enhanced acquired lytic activity against the parasite. In contrast, most coho salmon (*Oncorhynchus kisutch*) from a resistant stock survived up to three challenges with *C. salmositica*, without "immunization" at elevated temperatures. However, they acquired little or no lytic activity against the parasite, which survived in low numbers in some fish. Also, the chinook and coho salmon did not have innate plasma factors that lysed the parasite under in vitro conditions like those demonstrated in other salmonids. Thus, the mechanism(s) that protect the resistant *O. kisutch* from the pathogenic affects of *C. salmositica* are different from those identified in other fishes that are resistant to *Cryptobia* spp. A challenge of 10^5 flagellates per fish was suggested to be representative of the number of *C. salmositica* inoculated into a fish by one infected leech vector (*Piscicola salmositica*).

Résumé : Des juvéniles de saumon quinnat (*Oncorhynchus tshawytscha*) on résisté, grâce à un acclimatement à une température de l'eau élevée (20 °C), à une mise en présence de l'hémoflagellé *Cryptobia salmositica* qui aurait été mortelle dans d'autres conditions. Après une mise à l'épreuve à des températures favorables à l'hémoflagellé (9–11 °C), le plasma des poissons survivant présentait une activité lytique acquise accrue contre le parasite. Par contre, la plupart des saumons cohos (*Oncorhynchus kisutch*) appartenant à une souche résistante ont survécu à trois mises à l'épreuve de *C. salmositica*, sans « immunisation » à températures élevées. Toutefois, ces poissons n'ont acquis pratiquement aucune activité lytique contre le parasite, qui a survécu en nombres peu importants chez certains poissons. De plus, on n'a pas observé chez le quinnat et le coho des facteurs plasmatiques innés pouvant lyser le parasite dans des conditions in vitro, comme c'était le cas chez d'autres salmonidés. Ainsi, le ou les mécanismes qui protègent l'espèce résistante *O. kisutch* des effets pathogènes de *C. salmositica* sont différents de ceux qu'on a identifiés chez d'autres poissons qui sont résistants aux *Cryptobia* spp. La mise à l'épreuve avec 10^5 flagellés par poisson a été jugée représentative du nombre de *C. salmositica* inoculés à un poisson par un spécimen infecté de sangsue (*Piscicola salmositica*).

[Traduit par la Rédaction]

The haemoflagellate *Cryptobia salmositica* Katz, 1951 is a biflagellated protozoan parasite that is a lethal pathogen to some stocks of Pacific salmon (genus *Oncorhynchus*) on the west coast of North America (Wales and Wolf 1955; Mundie and Traber 1983; Bower and Margolis 1984*a*; Woo 1987). In the laboratory, stocks such as chinook salmon (*O. tshawytscha*) from the Big Qualicum River (Vancouver Island) and sockeye salmon (*O. nerka*) from the Fulton River (Skeena River system) suffered high mortalities at low exposure (100 flagellates inoculated intraperitoneally per fish). However, other stocks, such as sockeye salmon from Weaver Creek (Fraser River system) and coho salmon (*O. kisutch*) from the Big Qualicum River, experienced few mortalities, even at exposures of 10^6 flagellates per fish (Bower and Margolis 1984*a*). These extreme differences in susceptibility correlated with whether or not the

Received May 3, 1994. Accepted March 7, 1995.
J12808

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salmon stocks had a history of contact with the flagellate in nature. Specifically, resistant salmon were from stocks that spend about 1 year as juveniles in enzootic areas where the flagellate and its leech vector, *Piscicola salmositica* Meyer, 1946, normally occur, while susceptible salmon were from stocks that live outside the normal distribution of *C. salmositica* in British Columbia, or that spend less than 3 months in rivers where *C. salmositica* occurs (Bower and Margolis 1984*a*, 1984*b*).

At least two mechanisms of defence against *C. salmositica* were revealed in sockeye salmon from susceptible and resistant stocks (from Fulton River and Weaver Creek, respectively) during laboratory investigations (Bower and Evelyn 1988). (*i*) Sockeye salmon from susceptible stocks could acquire protective immunity if they were acclimated to elevated water temperatures (20°C) within 11 d of being inoculated with 10^5 *C. salmositica*, and the plasma of these salmon showed enhanced lytic activity against the parasite via a mechanism that was antibody and complement mediated. (*ii*) Sockeye salmon from the resistant stock, and to a lesser extent, some fish from the susceptible stock, had innate plasma factors that destroyed the parasite in vitro via a mechanism of complement-mediated lysis that was triggered through the activation of the alternate pathway (Bower and Evelyn 1988). Jones and Woo (1987) also demonstrated that another salmonid (rainbow trout, *Oncorhynchus mykiss*) was capable of acquiring protective immunity against *C. salmositica*, and Woo (1992) reported that plasma from some brook trout (*Salvelinus fontinalis*), which were refractory to experimental infection, lysed *C. salmositica* via the alternate pathway of complement activation. Mechanisms used by chinook and coho salmon to control the haemoflagellate have not been investigated. Thus, one purpose of the current study was to ascertain if susceptible (chinook salmon from the Big Qualicum River) and resistant species (coho salmon from the Big Qualicum River) can control *C. salmositica* via mechanisms similar to those used by other salmonids.

The optimum number of flagellates to use when challenging salmonids with *C. salmositica* to test for innate and acquired resistance in the laboratory has never been determined. To approximate conditions in nature, it would be justifiable to challenge the fish with a number of *C. salmositica* that they are likely to encounter in the wild. Although direct transmission of *C. salmositica* can occur in the wild under unusual circumstances (Bower and Margolis 1983), the usual method of transmission is via the leech vector *P. salmositica* (Becker and Katz 1965). Because the number of parasites that the leech is capable of administering to the fish on which it feeds is unknown, this study also estimates the number of *C. salmositica* one leech can administer to a juvenile salmon during 24 and 48 h of cohabitation.

Materials and methods

Chinook and coho salmon gametes were obtained from fish from the Big Qualicum River, Vancouver Island, B.C., Canada. All fertilized eggs and fish derived from them were held at the Rosewall Experimental Hatchery (Vancouver Island). Fish were reared for about 1 year in indoor tanks on a ground water supply and were fed a commercial diet of Oregon Moist Pellets®. Thus, none of the fish used in this study had prior exposure to *C. salmositica*. At the time of the experiments, the fish were 9–15 cm in fork length and 9–48 g in wet weight.

Sixty chinook salmon were vaccinated against *C. salmositica* using incubation in warmed water to give the fish survival advantage over the parasite as described for the vaccination of sockeye salmon (Bower and Evelyn 1988). Briefly, the fish were inoculated intraperitoneally with 10^5 *C. salmositica* per fish at 9°C and then acclimated to 20°C over a period of 14 d. They were maintained at 20°C from day 14 to 44 post-inoculation, a period sufficient to permit all 10 inoculated fish held at 9°C (initial controls) to succumb to the infection and for the majority of the fish held at 20°C (the vaccinates) to no longer show infections using the modified haematocrit centrifugation technique (Bower and Margolis 1984*a*). At this time total mortalities were calculated, the blood of nine vaccinates was examined for the parasite and for anti-*C. salmositica* activity, and the water temperature was reduced over 3 d to between 9 and 11°C. The remaining 50 vaccinates were then challenged with an intraperitoneal injection of 10^5 *C. salmositica* per fish. Controls ($n = 45$) used in conjunction with this challenge consisted of fish that had not previously been inoculated with the parasite; they were incubated at 20°C for the same period as the vaccinates, following which the water temperature was reduced and they were challenged as per the vaccinates.

Coho salmon ($n = 37$) were vaccinated by inoculation (as described above) and incubated at 13°C instead of 20°C. On day 70 the blood of 20 vaccinates was examined for *C. salmositica* and all but a random collection of eight fish were discarded. On day 270 and again on day 324, the eight fish were challenged at about 10°C by intraperitoneal inoculation of 10^5 and 8×10^5 *C. salmositica* per fish, respectively. The pathogenicity of the parasites used for vaccination on day 1 and for challenge on day 270 was tested in susceptible salmonids (chinook salmon ($n = 50$) and Fulton River Sockeye salmon ($n = 31$), respectively). A positive control was not used for the challenge on day 324.

The blood of fish sampled during and at the end of the experiments was examined using wet-mount and modified haematocrit centrifugation techniques (Bower and Margolis 1984*a*) to determine the intensity of infection. Parasitaemias were categorized as negative if no flagellates were observed, low if flagellates were observed by only the modified haematocrit centrifugation technique, and high if flagellates were observed by both techniques. The plasma (collected using 0.25 U sodium heparin/mL blood) from the vaccinated and challenged chinook and coho salmon was heat inactivated at 45°C for 20 min and stored at −20°C until required.

The antibody titre of vaccinated fish was determined using an immunolysis test. This test and tests for lytic activity in plasma of unvaccinated fish were performed as described by Bower and Evelyn (1988).

To estimate the number of haemoflagellates inoculated by the leech vector, susceptible chinook salmon (126.4 ± 14.0 mm in length and 20.3 ± 7.9 g in wet weight), which showed no innate lytic activity against *C. salmositica*,

were used in all assays. Sixteen fish were individually placed into 6-L pails containing 2 L of water and supplied with bubbling air. The pails were held in a water bath at about 10°C and covered to provide darkness and thus, quieten the fish. One moderate-sized leech (about 2.5 cm in length; average wet weight of 0.07 ± 0.04 g), from a river in which about 100% of the leeches were infected with *C. salmositica*, was added to each pail. After 24–48 h of cohabitation, each fish was assayed for *C. salmositica* by examining wet mounts of blood and by using the modified haematocrit centrifugation technique. The wet weight of the leech was determined to obtain a measure of its size, and its gut contents were examined for *C. salmositica* to assure that it was infected.

As criteria for evaluating the relationship between parasitaemias and inocula, the number of inoculated flagellates required to obtain detectable parasitaemias within 24 h (i.e., detection of *C. salmositica* in circulation with minimum time allowed for the parasite to multiply) was determined. Groups of three to six fish were inoculated intraperitoneally with dilutions of *C. salmositica* isolated from either heavily infected fish or from the gut contents of infected leeches (*P. salmositica*). About 24 h later, each fish was assayed for *C. salmositica* as described above.

Results

Acquired immune response to *Cryptobia salmositica*

Fifty-nine of the 60 chinook salmon vaccinated by inoculation with *C. salmositica* followed by acclimation to 20°C within 2 weeks were still alive by day 44. All 10 controls that were not transferred to 20°C but held at 9–11°C died with high parasitaemias by day 44. On day 46, only two of nine fish transferred to 20°C had detectable but low parasitaemias. The immunolysis antibody titres in five of the nine fish were undetectable in two fish, 1:4 in one fish, and 1:8 in two fish.

Seven of 50 vaccinated chinook salmon challenged at 9–11°C on day 47 survived to day 94 (47 d after challenge). In comparison, all 45 control fish (held at 20°C as per the vaccinated fish but without prior exposure to *C. salmositica*) died with high parasitaemias within 27 d of challenge. All seven surviving vaccinated fish had high parasitaemias on day 94. The immunolysis antibody titres were undetectable in one fish, 1:16 in two fish, 1:32 in three fish, and 1:64 in one fish.

The coho salmon demonstrated a response to *C. salmositica* that was different from that of the chinook salmon. Although the incubation temperature was low (13°C instead of 20°C used for the chinook), none of the 37 vaccinated coho salmon died by day 70. Only 7 of 53 controls held at about 10°C died. All dead control fish had high parasitaemias. Parasitaemias in the control survivors were undetectable in 24, low in 18, and high in 4 fish. The inoculum used during the vaccination experiment was verified as pathogenic because all 50 chinook salmon that were held at about 10°C died with high parasitaemias by day 29. Parasitaemias in 20 of the vaccinated coho salmon examined on day 70 were undetectable in nine, low in eight, and high in three fish. Nine other vaccinated fish were discarded, leaving eight to be challenged.

None of eight vaccinated coho salmon suffered mortalities following the first and second challenges on days 270 and 324, respectively. The inoculum used in the first challenge was proven pathogenic because all 31 susceptible Fulton River sockeye salmon (positive controls) died with high parasitaemias by day 35, and the inoculum used in the last challenge was eight times the number of *C. salmositica* used in the first challenge. On day 364 (40 d after the last challenge), parasitaemias in the vaccinated coho salmon were undetectable in five fish and low in three fish. In addition, only four of the eight fish had detectable immunolysis antibody titres (1:4 in three fish and 1:8 in one fish).

Innate immunity to *Cryptobia salmositica*

In two trials using plasma pooled from three or four chinook salmon and in three trials using plasma pooled from three to seven coho salmon, no innate lysis of *C. salmositica* was detected.

Number of haemoflagellates inoculated by the leech vector

Following cohabitation with the juvenile chinook salmon, all 16 leeches were infected with *C. salmositica*. Parasitaemias in the 11 fish cohabited with a leech for about 24 h were undetectable in five, low in four (from one to nine haemoflagellates at the buffy layer of each centrifuged capillary tube of blood and none detected in wet-mount blood preparations), and high in two fish (too many haemoflagellates to count in the centrifuged capillary tubes and from one to two haemoflagellates per 50 wet-mount microscopic fields (1850 μm in diameter) of blood). Parasitaemias in the five fish following cohabitation with a leech for about 48 h were low in two fish (1–52 haemoflagellates per centrifuged capillary tube of blood and none detected in wet-mount blood preparations) and high in two fish (too many haemoflagellates to count in the centrifuged capillary tubes and about two haemoflagellates per 50 wet-mount microscopic fields (1850 μm in diameter) of blood from one fish and three per 20 fields in blood from the other fish).

Haemoflagellates were detected in the blood of the chinook salmon 24 h after inoculation with *C. salmositica* (Table 1). In fish inoculated with blood forms, the numbers detected increased proportionally with the number inoculated such that numerous haemoflagellates were observed in the fish inoculated with one million *C. salmositica*. However, only a few *C. salmositica* were detected in fish inoculated with one million haemoflagellates that were isolated from the gut of the leech vector.

Discussion

The tendency for parasitaemias of *C. salmositica* not to develop, and for the fish to survive infection at warm temperatures (about 20°C) is well documented and is probably attributable to the sensitivity of the flagellate to elevated temperatures (Woo et al. 1983; Bower and Margolis 1985; Bower and Evelyn 1988). In the present study, the chinook salmon, protected from the lethal affects of *C. salmositica* by this warm-water treatment (vaccination), had reduced mortalities in comparison to unvaccinated fish

Table 1. Mean number of *Cryptobia salmositica* detected in preparations of fresh blood (wet mount) or by the modified haematocrit centrifugation technique (haematocrit) from *Oncorhynchus tshawytscha* 24 h after intraperitoneal inoculation with *C. salmositica* obtained from the blood of fish (blood forms) or from the gut of the vector, *Piscicola salmositica* (leech forms).

	Fish given blood forms			Fish given leech forms	
No. of parasites inoculated	No. of fish examined	Wet mount	Haematocrit	No. of fish examined	Haematocrit
1×10^3	4	0	2 (0–5)	5	0
5×10^3	4	0	3 (0–12)	0	—
1×10^4	4	0	74 (9–125)	4	0
5×10^4	4	0	TMTC[b]	0	—
1×10^5	4	3/20 fields[a]	TMTC	6	0
5×10^5	4	3/1 field	TMTC	0	—
1×10^6	3	5/1 field	TMTC	5	0.5 (0–3)

[a]Each microscope field had a diameter of about 1850 μm and was examined at low power (100×) magnification.

[b]There were too many haemoflagellates at the buffy layer of the capillary tube for a count to be made.

when subsequently challenged at cooler temperatures where infection is usually lethal for this fish. Although the survivors of the challenge had high parasitaemias, they also had immunolysis antibody titres that were at least a twofold increase over titres obtained from the vaccinated fish prior to challenge.

Generally, vertebrates with high parasitaemias have relatively low antibody titres because the antibodies form complexes with the parasite and (or) its secreted antigens, leaving few free antibody in the blood. Possibly, the chinook salmon in the present study had one or more limiting factors (e.g., component(s) in the complement activation pathway) that spared the haemoflagellates in circulation from destruction while allowing an increase in unbound antibodies. It was noted that haemoflagellates from these fish were unusually delicate and often lysed when smeared on slides to dry.

Protection (i.e., acquired resistance) against *C. salmositica* was also demonstrated in a susceptible stock of sockeye salmon following a similar procedure of immunization (Bower and Evelyn 1988). Like the chinook salmon, a high percentage (at least 40%) of the immunized sockeye salmon succumbed to infection on challenge. In both studies, the level of challenge (inoculation with 10^5 *C. salmositica*) was thought to represent natural levels of exposure delivered by vectors in the field (see below). Thus, the relatively low level of protection provided by this method of immunization and the difficulty involved in implementation would make this warm-water technique impractical for field use. Woo and Li (1990) demonstrated better protection from cryptobiosis in rainbow trout via vaccination with an in vitro culture of an attenuated strain of *C. salmositica*. However, the rainbow trout used in their work could be categorized as resistant to mortalities caused by *C. salmositica*, because none of the 10 non-immunized control fish that were challenged with 10^5 *C. salmositica* died. The efficacy of this attenuated parasite strain for immunization should be tested on susceptible stocks of salmon to evaluate the potential of its protective ability in the field.

In the present study, the coho salmon demonstrated a response to *C. salmositica* that was different from that of the chinook salmon (reported herein) and susceptible sockeye salmon (Bower and Evelyn 1988). Although the parasite was capable of surviving in some coho salmon, none of the vaccinated group died following two challenges. Even at lower temperatures (about 10°C) only 7 of the 53 coho salmon (initial controls) died, thereby substantiating that this stock of coho salmon was innately resistant to *C. salmositica* as reported by Bower and Margolis (1984*a*). In addition, only half of the vaccinated and challenged coho salmon developed detectable immunolysis antibody titres, which were low in comparison to those found in the chinook and sockeye salmon. Thus, the mechanism(s) for protection against *C. salmositica* used by the resistant coho salmon was unlike that identified in susceptible salmonids.

The differences in resistance to *C. salmositica* can be attributed to life-history characteristics of the salmon. The susceptible stock of sockeye salmon originated from the Fulton River (Skeena River watershed), where neither *C. salmositica* nor its leech vector occur (Bower and Evelyn 1988; Bower and Margolis 1984*b*). Thus, this stock has no known history of contact with the flagellate. The susceptible chinook salmon and resistant coho salmon were from the Big Qualicum River, where both *C. salmositica* and its leech vector occur. Although both species are exposed to *C. salmositica* as adults (Bower and Margolis 1984*b*), they spawn soon after entering the river, and physically deteriorate and die shortly thereafter. Thus, the infection usually has little effect on the adults. However, the life-history strategy of each species during its residence in the river as juveniles is quite different. Most juvenile chinook salmon leave the river within a few months of hatching, when they are still very small in size (about 4 cm fork length, see Healey 1991). If they are preyed upon by a leech during their short stay in fresh water, they usually die. The few leeches remaining in the river at that time of year (late winter) are relatively large (about 2.5 cm in length) in comparison to the small chinook salmon, and

are thus able to exsanguinate the small fish. Therefore, chinook salmon have virtually no contact with *C. salmositica* as juveniles. Conversely, coho salmon remain in the river for about 1 year and are exposed to infected leeches during the fall and winter, when they are large enough (about 10 cm fork length, see Sandercock 1991) to withstand blood loss to the leech. Consequently, the juvenile coho salmon are infected with *C. salmositica* by the leech. Thus, for coho salmon to survive in the Big Qualicum River, they had to acquire mechanism(s) of resistance against *C. salmositica*. However, the mechanism of resistance does not seem to rely upon the classical antibody-mediated immune response as demonstrated in other fish against various parasites (Woo 1992).

The lack of detectable innate lysis of *C. salmositica* in plasma from susceptible chinook salmon is consistent with observations reported by Bower and Evelyn (1988) for susceptible stocks of sockeye salmon that also had little or no innate lytic activity against this haemoflagellate. However, the lack of detectable innate lytic activity in the plasma from resistant coho salmon is unusual. Several studies have demonstrated that fresh plasma from a number of fish species known to be resistant to infection or resistant to mortalities caused by *Cryptobia* spp. contain detectable levels of innate lytic activity (Bower and Woo 1977; Wehnert and Woo 1980; Bower and Evelyn 1988). The mechanism of defence against *C. salmositica* that is displayed by the resistant coho salmon stock is not understood, but it may rely more heavily on a cellular response (Ellis 1977; McKinney et al. 1977) or other mechanisms (Ingram 1980; Fletcher 1981).

The prevalence and intensity of infection with *C. salmositica* were greater in the five chinook salmon cohabited with an infected leech for 48 h than in the 11 fish cohabited for 24 h. The lack of visible feeding wounds on the five uninfected fish cohabited for 24 h suggests that the leeches did not have sufficient time to attach and feed. Nevertheless, detectable parasitaemias in 11 fish indicate that a leech is capable of delivering numerous *C. salmositica* when it feeds. Although the parasitaemias were variable, as expected, five fish had high parasitaemias, with up to three haemoflagellates in 20 wet-mount microscopic fields (1850 μm in diameter) of blood. Similar parasitaemias were achieved by the intraperitoneal inoculation of 10^5 blood forms. However, blood forms are probably not homologous to the "metacyclic" forms delivered by the leech vector and the leech does not appear to deliver the parasites into the body cavity as achieved by intraperitoneal inoculation.

High parasitaemias were not obtained by the intraperitoneal inoculation of up to 10^6 forms obtained from infected leeches. Possibly the *C. salmositica* from the gut of the leech did not enter the circulatory system as readily as the blood forms isolated from fish, or maybe many of the *C. salmositica* isolated from the leeches were transformed dividing stages (see Lom and Dyková 1992, p. 55) that could not survive in the final host.

Although it may not be appropriate to compare parasitaemias resulting from the intraperitoneal injection of blood forms or leech gut forms of *C. salmositica* with those delivered by the leech as indicated above, this study provides some substantiation for the numbers of *C. salmositica* appropriate for experimental exposures. Thus, it seems reasonable to inoculate salmonids with about 1×10^5 *C. salmositica* when challenging them in the laboratory to test for innate and acquired resistance because in nature a salmon could easily acquire this number from an infected leech.

Acknowledgments

I thank Gary Meyer and Pamela David for their technical assistance in some of the experiments, and the personnel at Rosewall Experimental Hatchery, Vancouver Island, B.C., under the supervision of R.M. Humphreys, for rearing the salmon.

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Redescription of *Myxobolus rhinichthidis* (Myxosporea) parasitizing *Rhinichthys cataractae*, with a revised taxonomic list of species of *Myxobolus* known from North American freshwater fishes

David K. Cone and Richard L. Raesly

Abstract: *Myxobolus rhinichthidis* Fantham, Porter and Richardson, 1939 (Myxosporea) is redescribed from longnose dace (*Rhinichthys cataractae*) in Maryland. Large round plasmodia occurred in the connective tissue of the skin covering the head and opercular regions. Fixed spores are oval in front view and measure 11.5–14.5 μm long, 9.0–10.5 μm wide, and 6.0–7.0 μm thick. The polar capsules are 5.5–7.0 μm long and 3.0–3.5 μm wide. Photomicrographs of formalin-fixed spores document variation in spore size and shape. The report is the first for *M. rhinichthidis* since the species was described from blacknose dace (*Rhinichthys atratulus*) in Quebec. An updated list of species of *Myxobolus* Bütschli, 1882 reported from freshwater fishes in North America is provided. *Myxobolus jahnricei* Landsberg and Lom, 1991 is considered a junior synonym of *Myxobolus filamentus* Grinham and Cone, 1990; and *Myxobolus lii* Desser, 1993 and *Myxobolus gibbosus* Li and Desser, 1985 are junior synonyms of *Myxobolus uvuliferus* Cone and Anderson, 1977.

Résumé : Nous présentons une nouvelle description de la myxosporidie *Myxobolus rhinichthidis* Fantham, Porter et Richardson, 1939 à partir de spécimens prélevés sur des naseux de rapides (*Rhinichthis cataractae*) au Maryland. De gros plasmodes ronds étaient présents dans le tissu conjonctif de la peau couvrant la tête et les régions operculaires. Les spores fixées sont ovales vues de front et mesurent 11,5–14,5 μm de longueur, 9,0–10,5 μm de largeur et 6,0–7,0 μm d'épaisseur. Les capsules polaires mesurent 5,5–7,0 μm de longueur et 3,0–3,5 μm de largeur. Les microphotographies de spores fixées au formol révèlent la variation de la taille et de la forme des spores. C'est le premier rapport sur *M. rhinichthidis* depuis la description de l'espèce chez le naseux noir (*Rhinichthis atratulus*) au Québec. Nous mettons à jour la liste des espèces de *Myxobolus* Bütschli, 1882 signalées chez les poissons d'eau douce en Amérique du Nord. *Myxobolus jahnricei* Landsberg et Lom, 1991 est considéré comme le synonyme le plus récent de *Myxobolus filamentus* Grinham et Cone, 1990; *Myxobolus lii* Desser, 1993 et *Myxobolus gibbosus* Li et Desser, 1985 sont les synonymes les plus récents de *Myxobolus uvuliferus* Cone et Anderson, 1977.

[Traduit par la Rédaction]

One hundred and nine species of *Myxobolus* Bütschli, 1882 (Myxosporea) are known from freshwater fishes in North America. Most of these are inadequately described, particularly with respect to details of the fully developed spore, and many have not even been reported since the original species description earlier this century. One at a time these poorly understood parasites are being redescribed with the accuracy of photomicrography (Lom and Hoffman 1971; Cone and Anderson 1977; Desser and Paterson 1978; Cone 1979, 1983; Lom 1987; Beis and Cone 1990; Grinham and Cone 1990; Lom et al. 1992; Lom and Dykova 1992).

The present study redescribes *Myxobolus rhinichthidis* Fantham, Porter, and Richardson, 1939 from the longnose dace (*Rhinichthys cataractae*) in Maryland, and provides an updated list of species of the genus reported from North American freshwater fishes.

Received April 7, 1994. Accepted December 15, 1994
J12593

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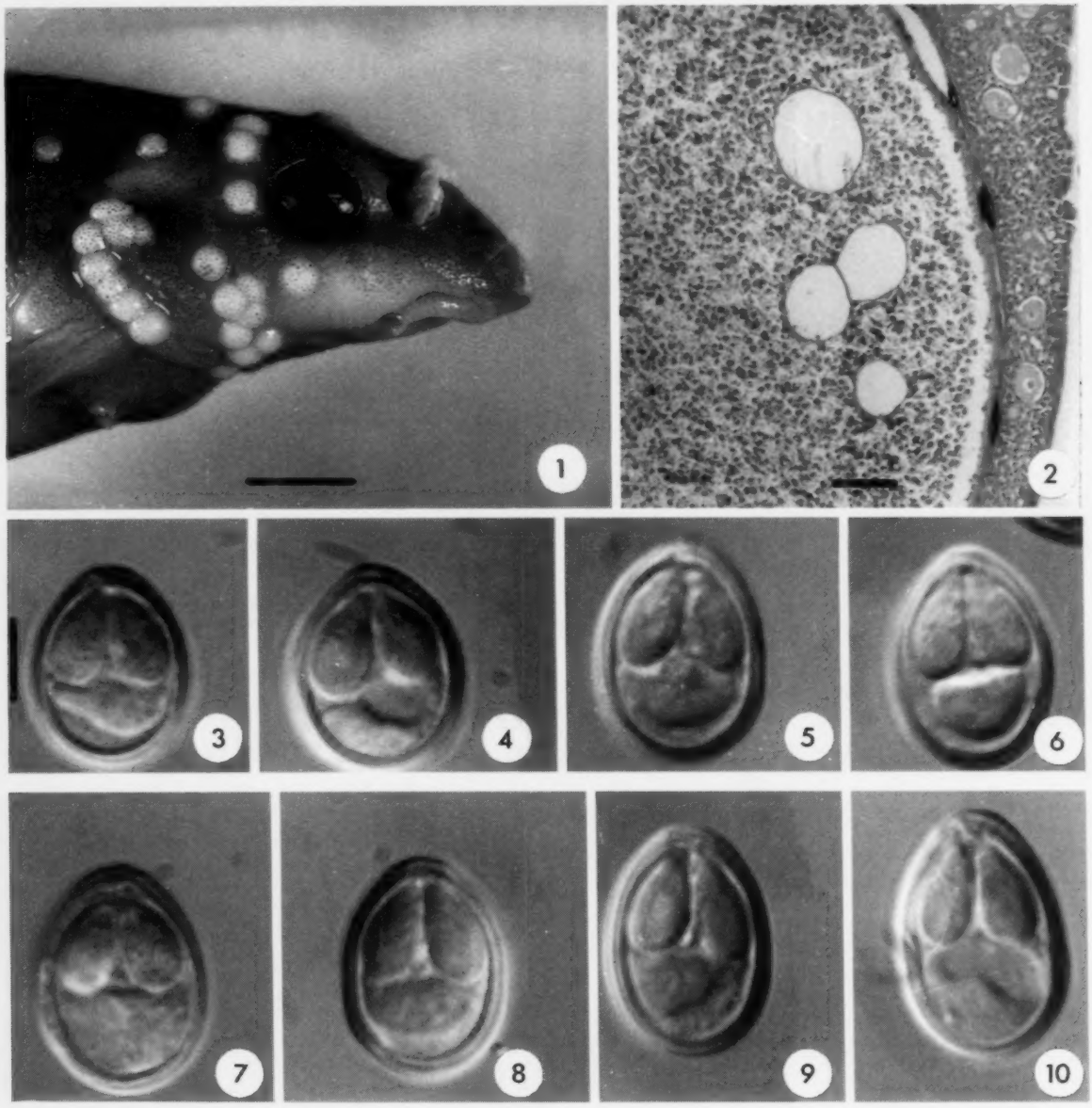
Materials and methods

An extensive ichthyofaunal survey of the Youghiogheny and Potomac river drainages in western Maryland was conducted between 1990 and 1993. Fishes were collected by electrofishing and seining from 73 sites in Garret, Allegany,

Fig. 1. Enlarged view of head of longnose dace (*Rhinichthys cataractae*) showing pseudocysts of *Myxobolus rhinichthidis* Fantham, Porter, and Richardson, 1939. Scale bar = 5 mm. **Fig. 2.** Histological section of plasmodium of *M. rhinichthidis* showing unidentified vessels (arrows) passing through the endoplasm. Scale bar = 100 μm. **Figs. 3–10.** Photomicrographs of formalin-fixed spores of *M. rhinichthidis* showing variation in spores size and shape. Interference contrast. Scale bar = 5 μm.



and Washington counties. Fishes were fixed in the field in 10% formalin and later transferred to 50% isopropanol. An intensely infected *R. cataractae* collected May 29, 1992, at Middle Forks of the Savage River, Garrett County, Maryland, forms the basis of this report.

Several pseudocysts were ruptured and the contents mounted on 1.5% agar for microscopical study of spores. Photomicrographs of 15 randomly chosen spores were used to calculate spore dimensions as well as record variation in spore shape. Other pseudocysts were used to prepare histological sections for staining with hematoxylin and eosin. Unless otherwise stated, measurements are presented in micrometres as the mean ± 1 standard deviation followed by ranges in parentheses.

Results

Myxobolus rhinichthidis Fantham, Porter and Richardson, 1939 Figs. 1–11

Redescription

Pseudocyst white with speckles of melanocytes (Fig. 1). Plasmodium round, 1.5–2.0 mm in diameter, with thin peripheral ectoplasm and central endoplasm containing fully developed spores within a loose debris. Large, unidentified vessels passing through endoplasm (Fig. 2). Plasmodia seated within the loose connective tissue of the dermis but protruding above skin surface while remaining covered by slightly thinned epidermis.

Formalin fixed spore oval in front view (Figs. 3–11), 12.9 ± 0.8 (11.5–14.5) (n = 15) long, 9.5 ± 0.4 (9–10.5) wide, 6.5 ± 0.3 (6.0–7.0) thick. Polar capsules pyriform, 6.5 ± 0.5 (5.5–7.0) long, 3.1 ± 0.2 (3.0–3.5) wide. Spore length to width ratio 1.35 ± 0.10. Eight filament coils, wound loosely in polar capsule; coils angled 45° to longitudinal plane of polar capsule (Fig. 11). Ratio of polar capsule length to spore length 0.49 ± 0.02. Intercapsular appendix and sutural ridge folds absent.

Host: Longnose dace (*Rhinichthys cataractae*). The infected dace was 75 mm long, female, and 2+ years old.

Locality: Middle Fork of Savage River, Garrett County, Maryland.

Location: Twenty-six plasmodia on head and adjacent nape and thoracic regions. They were distributed asymmetrically over these surfaces; 21 of 26 occurred in the skin of postocular, opercular, and subopercular areas of the right side of the head.

Prevalence of infection: Two of 638 *R. cataractae* were infected.

Specimens: Voucher specimens (Geimsa-stained smears) have been deposited in the United States National Museum, Beltsville, Maryland.

Comments

Myxobolus rhinichthidis was described originally from blacknose dace (*Rhinichthys atratulus*; syn.: *R. atronasus*) collected from a tributary of the Nicolet River, Ford Village, Quebec (Fantham et al. 1939). Round plasmodia measuring 2 mm in diameter were present on the head of the fish. Spores were oval and 8.6–11.8 μm long and 5.9–8.2 μm wide. Sutural ridge folds were apparently absent. The polar capsules were 3.6–5.5 μm long and 1.8–2.7 μm wide and less than half the length of the spore. The polar filament was loosely coiled within the capsule.

We do not know the exact method by which Fantham et al. (1939) prepared spores of *M. rhinichthidis* for study. They appear to have stained air-dried smears with one of many listed histological dyes. This would account for the distorted shapes of the depicted spores and the extreme detail with which the spore nuclei are described.

The present material was identified as *M. rhinichthidis* on the basis of several criteria. The parasite forms distinct, round plasmodia on the head and adjacent regions of the host and the spores are oval with the polar filaments coiled loosely in the capsule. The length of the polar capsules is less than half that of the spore length. The spore dimensions of *M. rhinichthidis* given by Fantham et al. (1939) are slightly less than those reported herein. However, these differences can be attributed to the different methods of preparing the spores for study. The parasite is known only from fishes of the genus *Rhinichthys* and the original report and the present are both from hosts collected from riverine environments in northeastern North America.

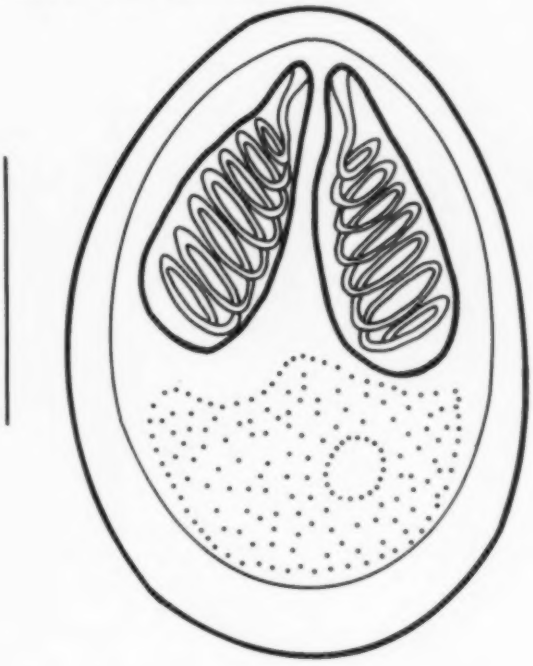


Fig. 11. Schematic drawing of spore of *M. rhinichthidis* (front view). Scale bar = 5 μm.

Between 1990 and 1993 we collected 11 945 fishes representing eight families and 44 species from Allegany, Garrett, and Washington counties in western Maryland. Of 3450 cyprinids collected from the Youghiogheny and Potomac river drainages 2169 were *R. atratulus* and *R. cataractae*. Pseudo-cysts of *M. rhinichthidis* were found on only two individuals from this large sample: the *R. cataractae* specimen reported above and another *R. cataractae* collected from Big Run in Garrett County (Potomac River system, Savage River drainage). Thus the parasite is host specific to fishes of the genus *Rhinichthys*, and it is very rare in the studied drainages, the prevalence of infection being less than 0.08% in these host populations.

Discussion

Lom and Noble (1984) synonymized the genera *Myxobolus* Bütschli, 1882 and *Myxosoma* Thélohan, 1892. The result of this amalgamation involving 445 species created a taxonomic nightmare because *Myxosoma* is a Greek feminine

noun and *Myxobolus* is a Latin masculine noun. Thus the appropriate suffix changed on the specific epithets. Furthermore, many specific names applied to valid taxa in *Myxosoma* became homonyms of the same specific names in *Myxobolus*. In an attempt to correct this situation, Grinham and Cone (1990) changed the names of several species of *Myxobolus* when reviewing members of the genus parasitizing catostomid fishes. However, in a taxonomic review of all species of *Myxobolus*, Landsberg and Lom (1991) made the same corrections, duplicating some of those already made by Grinham and Cone (1990). The following final revisions are in order.

Myxobolus okobojiensis Otto and Jahn, 1943 was described from *Pomoxis spariodes* and *Myxosoma okobojiensis* Rice and Jahn, 1943 from *Ictiobus bubalis*. When the two genera were synonymized a homonym was created. Grinham and Cone (1990) established a new species, *Myxobolus filamentus* Grinham and Cone, 1990 for the former *Myxosoma okobojiensis*. Almost at the same time, Landsberg and Lom (1991) created *Myxobolus jahnricei* for the same homonym. *Myxobolus jahnricei* Landsberg and Lom, 1991 is thus a junior synonym of *Myxobolus filamentus* Grinham and Cone, 1990.

The transfer of *Myxosoma rotundum* Meglitsch, 1937 to *Myxobolus* creates *Myxobolus rotundus* (Meglitsch, 1937), but this is a homonym of *Myxobolus rotundus* Nemeczek, 1911. Grinham and Cone (1990) created *Myxobolus meglitschi* to avoid the homonym. Landsberg and Lom (1991) created the same species; thus the correct name for this species is *Myxobolus meglitschi* Grinham and Cone, 1990.

Li and Desser (1985) described *Myxobolus gibbosus* from *Lepomis gibbosus*, a homonym of *Myxobolus gibbosus* Herrick, 1941 from the same host. Realizing the duplication in names, Desser (1993) established *Myxbolus lii* to replace *M. gibbosus* Li and Desser, 1985. However, Landsberg and Lom (1991) had previously synonymized *M. gibbosus* Li and Desser, 1985 with *Myxbolus uvuliferis* Cone and Anderson, 1977. Thus, *Myxobolus lii* Desser, 1993 is also a junior synonym of *Myxobolus uvuliferus*.

To assist subsequent workers, an updated list of species of *Myxobolus* known from freshwater fishes in North America is presented below:

Myxobolus angustus Kudo, 1934
M. arcticus Pugachev and Khokhlov, 1979
M. argenteus Lewis, 1968
M. artus Akhmerov, 1960
M. aureatus Ward, 1919
M. bellus Kudo, 1934
M. bibullatus (Kudo, 1934)
  Syn.: *Myxosoma bibullatum* Kudo, 1934
M. bilineatum Bond, 1938
M. bondi Landsberg and Lom, 1991
  Syn.: *Myxosoma muelleri* Bond, 1939
M. bubalis Otto and Jahn, 1943
M. capsulatus Davis, 1917
M. cartilaginis (Hoffman, Putz and Dunbar, 1965)
  Syn.: *Myxosoma cartilaginis* Hoffman, Putz and Dunbar, 1965
M. catostomi (Kudo, 1923)
  Syn.: *Myxosoma catostomi* Kudo, 1923; *Myxobolus catostomi* Fantham, Porter and Richardson, 1939
M. cerebralis Hofer, 1903
  Syn.: *Lentospora cerebralis* (Hofer, 1903); *Myxosoma cerebralis* (Hofer, 1903)
M. commersonii (Fantham, Porter and Richardson, 1939)
  Syn.: *Myxosoma commersonii* Fantham, Porter and Richardson, 1939
M. compressus Kudo, 1934
M. congesticus Kudo, 1934
M. conspicuus Kudo, 1929
M. corneus Cone, Horner, and Hoffman, 1990
M. couesii Fantham, Porter and Richardson, 1939
M. cuneatus (Bond, 1939)
  Syn.: *Myxosoma cuneata* Bond, 1939
M. cyprinicola Reuss, 1906
M. dechtiari Cone and Anderson, 1977
M. dentium Fantham, Porter and Richardson, 1939
M. diaphanus (Fantham, Porter and Richardson, 1940)
  Syn.: *Myxosoma diaphana* Fantham, Porter and Richardson, 1940
M. discrepans Kudo, 1920
M. dujardini (Thélohan, 1892)
  Syn.: *Myxosoma dujardini* Thélohan, 1892
M. ellipticoides (Fantham, Porter and Richardson, 1939)
  Syn.: *Myxosoma ellipticoides* Fantham, Porter and Richardson, 1939
M. endovasus (Davis, 1947)
  Syn.: *Myxosoma endovasa* Davis, 1947
M. eucalii (Guilford, 1965)
  Syn.: *Myxosoma eucalii* Guilford, 1965
M. fanthami Landsberg and Lom, 1991
  Syn.: *Myxobolus grandis* Fantham, Porter and Richardson, 1939
M. filamentus Grinham and Cone, 1990
  Syn.: *Myxosoma okobojiensis* Rice and Jahn, 1943; *Myxobolus janricei* Landsberg and Lom, 1991
M. funduli Kudo, 1920
  Syn.: *Myxobolus musculi* Hahn, 1915; note that *Myxosoma funduli* Kudo, 1918 is a junior synonym of *Myxobolus cyprini* Doflein, 1898
M. gibbosus Herrick, 1941
M. globosus Gurley, 1893
M. grandis (Kudo, 1934)
  Syn.: *Myxosoma* grandis Kudo, 1934
M. gravidus Kudo, 1934
M. hendricksoni Mitchell, Seymour and Gamble, 1985
M. heterolepis Li and Desser, 1985
M. hoffmani (Meglitsch, 1963)
  Syn.: *Myxosoma hoffmani* Meglitsch, 1963
M. hudsonis (Bond, 1938)
  Syn.: *Myxosoma hudsonis* Bond, 1938
M. hyborhynchi Fantham, Porter and Richardson, 1939
M. inornatus Fish, 1938
M. insidiosus Wyatt and Pratt, 1963
M. intestinalis Kudo, 1929
M. iowensis Otto and Jahn, 1943
M. kisutchi Yasutake and Wood, 1957
M. koi Kudo, 1920
M. kostiri Herrick, 1936
M. lamellus Grinham and Cone, 1990

M. lepomicus Li and Desser, 1985
M. lintoni Gurley, 1893
M. magnaspherus Cone and Anderson, 1977
M. medius (Fantham, Porter and Richardson, 1939)
Syn.: *Myxosoma media* Fantham, Porter and Richardson, 1939
M. meglitschi Grinham and Cone, 1990
Syn.: *Myxosoma rotundum* Meglitsch, 1937
M. mesentericus Kudo, 1920
M. microcystus Price and Mullen, 1980
M. microlatus Li and Nie, 1973
M. microthecus (Meglitsch, 1942)
Syn.: *Myxosoma microthecus* Meglitsch, 1942
M. moxostomi Nigrelli, 1948
M. muelleri Bütschli, 1882
Not to be confused with the homonym *M. muelleri* Bond, 1939, a synonym of *M. bondi* Landsberg and Lom, 1991
M. multiplicatus (Reuss, 1906)
Syn.: *Lentospora multiplicatus* Reuss, 1906
M. musculi Keysselitz, 1908
Not to be confused with *Myxobolus musculi* Hahn, 1915, a junior synonym of *Myxobolus funduli*
M. mutabilis Kudo, 1934
M. nemachili Weiser, 1949
M. neurobius Schuberg and Schröder, 1905
M. neurophilus (Guilford, 1965)
Syn.: *Myxosoma neurophila* Guilford, 1965
M. nodosus Kudo, 1934
M. notemigoni Lewis and Summerfelt, 1964
M. notropis (Fantham, Porter and Richardson, 1939)
Syn.: *Myxosoma notropis* Fantham, Porter and Richardson, 1939
M. obliquus Kudo, 1934
M. oblongus Gurley, 1893
M. okobojiensis Otto and Jahn, 1943
M. orbiculatus Kudo, 1920
M. orbitalis (Fantham, Porter and Richardson, 1939)
Syn.: *Myxosoma orbitalis* Fantham, Porter and Richardson, 1939
M. osburni Herrick, 1936
M. ovalis (Davis, 1923)
Syn.: *Myxosoma ovalis* Davis, 1923
M. ovatus Kudo, 1934
M. ovoidalis Fantham, 1930
M. paraellipticoides (Fantham, Porter and Richardson, 1939)
Syn.: *Myxosoma paraellipticoides* Fantham, Porter and Richardson, 1939
M. paralintoni Li and Desser, 1985
M. pellicides Li and Desser, 1985
M. pendula (Guilford, 1967)
Syn.: *Myxosoma pendula* Guilford, 1967
M. percae Fantham, Porter and Richardson, 1939
M. pfrille Fantham, Porter and Richardson, 1939
Syn.: *Myxosoma pfrille* Fantham, Porter and Richardson, 1939
M. pharyngeus (Parker, Spall and Warner, 1971)
Syn.: *Myxosoma pharyngeus* Parker, Spall and Warner, 1971
M. poecilichthidis Fantham, Porter and Richardson, 1939
M. pratti (Wyatt, 1979)
Syn.: *Facieplatycauda pratti* Wyatt, 1979
M. procerus (Kudo, 1934)
Syn.: *Myxosoma procerum* Kudo, 1934
M. pseudokoi Li and Desser, 1985
M. rhinichthidis Fantham, Porter and Richardson, 1939
M. robustus (Kudo, 1934)
Syn.: *Myxosoma robustum* Kudo, 1934
M. scardinii Reuss, 1906
M. schuberti Li and Desser, 1985
M. scleroperca (Guilford, 1963)
Syn.: *Myxosoma scleroperca* Guilford, 1963
M. spalli Landsberg and Lom, 1991
Syn.: *Myxosoma cyprini* Spall, 1974
M. sparoides Otto and Jahn, 1943
M. squamae Keysselitz, 1908
M. squamalis (Iversen, 1954)
Syn.: *Myxosoma squamalis* Iversen, 1954
M. squamosus Kudo, 1934
M. subcircularis Fantham, Porter and Richardson, 1939
M. subtecalis (Bond, 1939)
Syn.: *Myxosoma subtecalis* Bond, 1939
M. symmetricus Rice and Jahn, 1943
M. teres Kudo, 1934
M. transovalis Gurley, 1893
M. transversalis Fantham, Porter and Richardson, 1939
M. uvuliferus Cone and Anderson, 1977
Syn.: *Myxobolus gibbosus* Li and Desser, 1985; *Myxobolus lii* Desser, 1993
M. vastus Kudo, 1934
M. wellerae Li and Desser, 1985

Acknowledgements

This research was funded by grants 17129 and 314683 from the Maryland Natural Heritage Program, Maryland Department of Natural Resources (R.L.R.), and by an NSERC operating grant (D.K.C.).

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Proliferative kidney disease and *Sphaerospora oncorhynchi* in wild-caught salmonids from the Puntledge River system, Vancouver Island, British Columbia

M.L. Kent, M. Higgins, D.J. Whitaker, and H. Yokoyama

Abstract: Proliferative kidney disease (PKD), caused by the PKX myxosporean, was observed in kokanee salmon (non-anadromous sockeye salmon) (*Oncorhynchus nerka*) and chinook salmon (*O. tshawytscha*) collected from the Puntledge River, Vancouver Island, British Columbia in July 1993. This is the first report of a natural infection of PKX in either captive or wild sockeye salmon. All 14 underyearling kokanee salmon and the one underyearling chinook salmon exhibited numerous PKX organisms and associated chronic inflammation in the renal interstitium. Thirty-eight percent of sexually mature kokanee salmon collected in the autumn of 1993 from Comox Lake (which drains into the Puntledge River) were infected with *Sphaerospora oncorhynchi*, Kent, Whitaker and Margolis, 1993 in the renal tubules, while immature cutthroat trout (*O. clarki*) and coho salmon (*O. kisutch*) from the same collection did not exhibit myxosporean spores in the kidney. The kidneys of threespine stickleback (*Gasterosteus aculeatus*) and prickly sculpin (*Cottus asper*) collected from the Puntledge River during the summer were all infected with a *Myxobilatus* sp., but no organisms suggestive of PKX or *Sphaerospora* were observed. This study further supports the hypothesis that PKX is a developmental stage of *S. oncorhynchi*, which may sporulate only in sexually mature salmonids.

Résumé : La maladie proliférative du rein, causée par la myxosporidie PKX, a été observée chez des spécimens de kokani (saumon rouge non anadrome) (*Oncorhynchus nerka*) et de quinnat (*O. tshawytscha*) prélevés dans la rivière Puntledge, dans l'île de Vancouver, en juillet 1993. C'est la première fois qu'on signale une infection naturelle au PKX chez des saumons sauvages ou en captivité. Les 14 kokanis de moins d'un an et le quinnat du même âge présentaient de nombreux organismes PKX, ainsi que l'inflammation chronique du tissu interstitiel rénal qui est associée à leur présence. Chez les kokanis sexuellement matures prélevés à l'automne 1993 dans le lac Comox (dont la Puntledge est l'exutoire), 38% étaient infectés par *Sphaerospora oncorhynchi* Kent, Whitaker et Margolis, 1993 dans les tubules rénaux, tandis que les immatures de truite fardée (*O. clarki*) et de coho (*O. kisutch*) prélevés en même temps ne présentaient pas de spores de myxosporidies dans les reins. Les reins des épinoches à trois épines (*Gasterosteus aculeatus*) et des chabots piquants (*Cottus asper*) recueillis dans la Puntledge pendant l'été étaient tous infectés par un *Myxobilatus* sp., mais aucun organisme ressemblant au PKX ou au *Sphaerospora* n'a été observé. Notre étude vient conforter l'hypothèse selon laquelle le PKX serait un stade de développement de *S. oncorhynchi*, qui semble ne sporuler que chez les salmonidés sexuellement matures.

[Traduit par la Rédaction]

Received March 2, 1994. Accepted April 27, 1994.
J12521

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Introduction

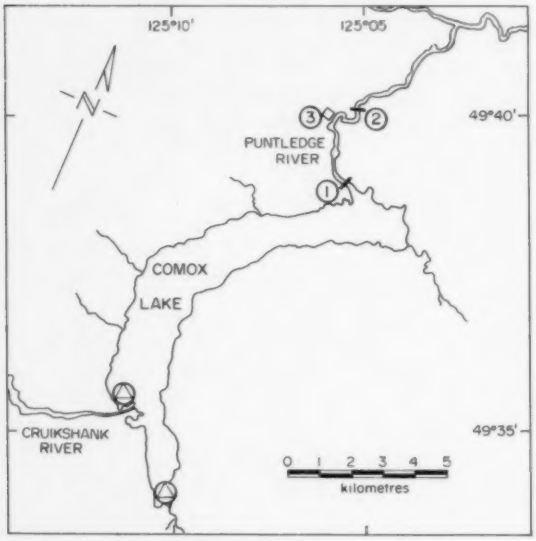
Proliferative kidney disease (PKD), caused by the PKX myxosporean, is an important disease of captive underyearling salmonids in North America and Europe (Clifton-Hadley et al. 1984; Hedrick et al. 1993). Although its identity has yet to be established, PKX may be an extrasporogonic stage of a *Sphaerospora*, *Parvicapsula*, or a related species (Kent and Hedrick 1986; Hedrick et al. 1988, 1993; Kent et al. 1993*b*; Landsberg 1993; Voronin and Chernysheva 1993). Because only immature spores of the

Table 1. Prevalence of renal myxosporeans in salmonids and non-salmonids collected in the Puntledge River system, 1992–1993.

Date	Fish species	Myxosporean species	Prevalence[a]	Age
12, 13 July 1992	Stickleback	*Myxobilatus* sp.	10/10	Unknown
26 July 1993	Kokanee	PKX	14/14	Underyearling
	Chinook	PKX	1/1	Underyearling
10 Aug. 1993	Stickleback	*Myxobilatus* sp.	9/9	Unknown
10 Aug. 1993	Sculpin	*Myxobilatus* sp.	6/6	Unknown
18 Aug. 1993	Stickleback	*Myxobilatus* sp.	3/3	Unknown
18 Aug. 1993	Sculpin	*Myxobilatus* sp.	1/1	Unknown
17 Nov. 1993	Kokanee	*S. oncorhynchi*	5/13	3–4 years, mature
17 Nov. 1993	Cutthroat	Unidentified intraluminal trophozoites	1/32	1–3 years, immature
17 Nov. 1993	Coho	No myxosporeans observed	0/7	Underyearling

[a]Number of positive/number examined.

Fig. 1. Puntledge River system, Vancouver Island, British Columbia. 1, impoundment dam; 2, diversion dam; 3, Puntledge River Hatchery (upper site). ⌂, location of gill net collections in Comox Lake.



parasite are found in clinically affected fish or in those that have recently recovered from the disease (Kent and Hedrick 1986, 1987; Hedrick et al. 1988; Clifton-Hadley and Feist 1989), the identity of PKX has not been conclusively determined. These observations suggest that salmonids may be aberrant hosts for PKX and that a non-salmonid fish may be the reservoir of infection (Seagrave et al. 1980; Kent and Hedrick 1986; Hedrick et al. 1988). However, attempts to link non-salmonid fishes to the infection have been unsuccessful (Hedrick et al. 1993; Kent et al. 1994), and Kent et al. (1993*b*) proposed that the PKX organism may be an extrasporogonic stage of *Sphaerospora oncorhynchi* Kent, Whitaker and Margolis, 1993, a myxosporean that usually sporulates only in sexually mature salmonids.

Although most reports of PKD are from hatchery-reared fish, there are a few reports of PKX and associated lesions in wild salmonids. The infection has been observed in wild-caught cutthroat trout (*Oncorhynchus clarki*) (MacConnell and Peterson 1992), chinook salmon (*O. tshawytscha*) (Hedrick et al. 1993), brown trout (*Salmo trutta*) (Wootten and McVicar 1982; Bucke et al. 1991), grayling (*Thymallus thymallus*), and pike (*Esox lucius*) (Seagrave et al. 1981; Bucke et al. 1991).

The Puntledge River Hatchery, Vancouver Island, B.C., has experienced several epizootics of PKD (Hoskins and Kieser 1986). We conducted the following survey of wild salmonid and non-salmonid fishes from the Puntledge River system in an attempt to identify reservoirs for PKX and to further elucidate the association of PKX with *S. oncorhynchi*.

Materials and methods

Fishes were collected from the Puntledge River near the hatchery with minnow traps or from a trap at a dam near the upper site of the Puntledge River Hatchery (Fig. 1) during the summer of 1992 and 1993 (Table 1). Fishes were collected from Comox Lake (Fig. 1) in the autumn of 1993 with gill nets in an attempt to identify PKX spores (e.g., *S. oncorhynchi*).

Whole fish or kidneys were preserved in Davidson's solution (Humason 1979), processed for histology, and slides were stained with hematoxylin and eosin. In addition, wet-mount preparations of the kidneys were examined from fishes collected 17 November 1993.

Results

Proliferative kidney disease was detected in all 14 kokanee salmon and the one chinook collected in July 1993 (Table 1). Numerous parasites and interstitial hyperplasia due to chronic inflammation were observed in the kidneys of all of these fish (Fig. 2).

Fig. 2. Proliferative kidney disease in underyearling kokanee salmon. Arrows indicate PKX parasites in kidney interstitium. Hematoxylin and eosin. Scale bar = 20 μm.

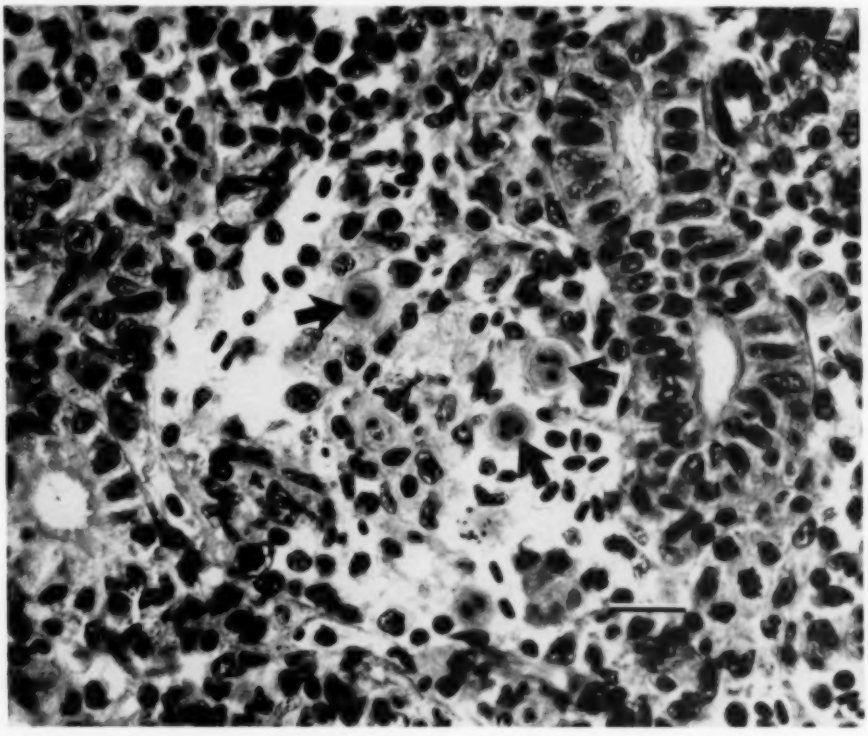


Fig. 3. *Sphaerospora oncorhynchi* in renal tubules of sexually mature kokanee salmon. Arrows indicate spores. Hematoxylin and eosin. Scale bar = 20 μm.

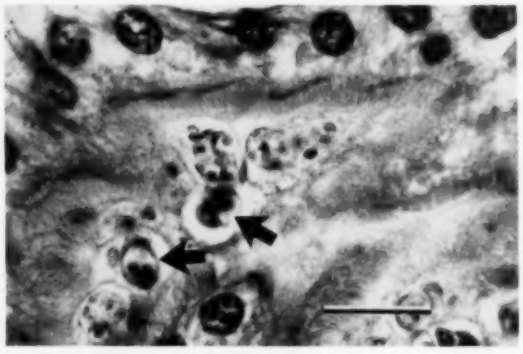
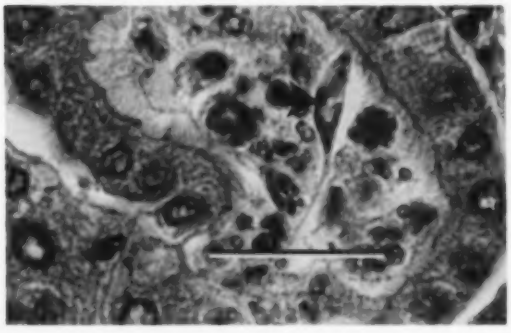


Fig. 4. *Myxobilatus* sp. in renal tubules of threespine stickleback. Arrow indicates spore. Hematoxylin and eosin. Scale = 20 μm.



Spores and trophozoites of *Sphaerospora oncorhynchi* were observed in the lumen of 38% of the kokanee salmon collected from Comox Lake in the autumn (Fig. 3). All of these fish were in spawning condition and had fully developed gonads. Intraluminal trophozoites were detected in one cutthroat trout, which was immature. Spores were not observed in this fish, and thus the identity of this myxosporean was not determined.

PKX-like organisms were not observed in prickly sculpin and threespine stickleback. However, *Myxobilatus* sp. (probably *M. gasteroistei* (Parisi, 1912)) trophozoites and spores were observed in the lumen of renal tubules of all these fishes (Fig. 4).

Discussion

This is the first report of PKD in naturally infected *Oncorhynchus nerka* (either sockeye or kokanee salmon). The intensity of the infection, severity of the associated inflammatory changes, and 100% prevalence of infection suggest that PKD has some impact on this population of

kokanee. However, the sampling method was probably biased toward collecting debilitated fish, which may had involuntarily been washed out of Comox Lake. Juvenile kokanee occasionally undergo seaward migration and thus become anadromous sockeye, but this phenomenon usually occurs at the time of normal seaward migration of sockeye salmon (i.e., May or June) (K. Hyatt, Pacific Biological Station, Nanaimo, B.C., personal communication). Because only one chinook underyearling was examined, we cannot speculate on the importance of PKD in wild chinook in this watershed. This fish was probably a hatchery-reared fish that had been released in the Cruikshank River (Fig. 1) early that year.

Observation of *S. oncorhynchi* in adult kokanee from the same watershed further supports our hypothesis that PKX and *S. oncorhynchi* are the same organism. Experimental transmission studies, field observations, and lectin and monoclonal staining have suggested that threespine stickleback, cottids (e.g., the Shoshone sculpin, *Cottus greenei*) and cyprinids (e.g., common carp, *Cyprinus carpio*, and the tui chub, *Gila bicolor*) are not reservoirs for PKX (Rafferty 1986; Rafferty and Mulcahy 1988; MacConnel and Smith 1990; Bucke et al. 1991; Hedrick et al. 1993; Kent et al. 1994), and our results concur with this supposition. Nevertheless, recent observations on common carp support the argument that PKX is a *Sphaerospora* species. Voronin and Chernysheva (1993) described an extrasporogonic form of *S. renicola* Dyková and Lom, 1982 in the gills of carp that was identical to PKX at an ultrastructural level, including the presence of identical "haplosporosomes" in enveloping cells. Possibly PKX-like cells occur in the development of several members of the genus *Sphaerospora*.

Aside from one rainbow trout (Hedrick et al. 1988), fully formed spores of *S. oncorhynchi* have only been detected in sexually mature sockeye salmon (Kent et al. 1993*b*) and sexually mature cutthroat trout (Hedrick et al. 1993). Identification of the *Sphaerospora* sp. in the rainbow trout and cutthroat trout as *S. oncorhynchi* was presumptive because only histological sections were available (Kent et al. 1993*b*). Kent et al. (1993*b*) proposed that sexual maturation may trigger sporulation of this myxosporean. Whereas fully formed spores of *S. oncorhynchi* were observed in many of the sexually mature kokanee, none were found in immature cutthroat trout from the same collection.

In conclusion, current evidence indicates that PKX (at least as found in North America) is a developmental stage of *S. oncorhynchi* for the following reasons: (*i*) both organisms have been found in salmonids within the same watershed at four separate locations; (*ii*) the intraluminal forms of PKX (Kent and Hedrick 1986; Hedrick et al. 1988) are indistinguishable from presporogonic forms of *S. oncorhynchi* described by Kent et al. (1993*b*); (*iii*) *S. oncorhynchi* stains positive with the *Griffonia simplicifolia* (GS-1) lectin (Kent et al. 1993*a*; E. MacConnell, U.S. Fish and Wildlife Service, Bozeman, MT, U.S.A., personal communication), a lectin that reacts strongly with the interstitial and intraluminal forms of PKX (Marin de Mateo et al. 1993); and (*iv*) attempts at identifying non-salmonid reservoir for PKX have thus far been unsuccessful.

Laboratory transmission studies with sockeye salmon and comparisons of PKX and *S. oncorhynchi* at the DNA level are underway or planned to further establish the relationship of the two organisms.

Acknowledgements

We thank the staff of the Puntledge River Hatchery for assistance. This study was partially funded by the Department of Fisheries and Oceans Salmon Enhancement Program.

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Effects of rearing conditions on growth and mortality of juvenile chum salmon (*Oncorhynchus keta*) infected with *Ichthyobodo necator*

Shigehiko Urawa

Abstract: Chum salmon (*Oncorhynchus keta*) juveniles, experimentally infected with the bodonid flagellate *Ichthyobodo necator* (Henneguy, 1883), were reared in 18-L tanks at three densities (300, 600, and 1200 fish) for 5 weeks. Water inflow rates were adjusted to give five combinations of crowding and flow rate. The parasite density on the fish body surface increased to 2700–4100 parasites·mm^{-2} over the course of the experiment and did not differ significantly among the groups. Total mortality was 76–90% in infected groups kept under the most unfavorable conditions (crowding or low water supply) but 11–15% in the other infected groups. The former groups also showed apparent growth reductions. In the uninfected controls, mortality was low (0.7–2.6%) and the degree of crowding or water supply had no significant effect on growth or mortality. Infected salmon were also much less resistant to seawater challenge; all fish kept at high stocking density or low inflow rate died in seawater. These results indicate that *Ichthyobodo* infections have marked effects on both host growth and survival when infections are combined with environmental stress induced by overcrowding and (or) inadequate water supply, although the parasite density is not affected by these factors.

Résumé : Des juvéniles de saumon kéta (*Oncorhynchus keta*), infestés expérimentalement avec le flagellé bodonidé *Ichthyobodo necator* (Henneguy, 1883), ont été élevés dans des aquariums de 18 L à trois densités différentes (300, 600 et 1200 poissons) pendant 5 semaines. L'entrée d'eau était ajustée de de façon à donner cinq combinaisons de densité de peuplement et de débit. La densité des parasites à la surface des poissons a augmenté pour atteindre 2700–4100 parasites·mm^{-2} pendant le cours de l'expérience, et ne différait pas de façon significative entre les groupes. La mortalité totale était de 76–90% dans les groupes infestés maintenus dans les conditions les plus défavorables (surpeuplement ou faible apport d'eau), mais de 11–15% dans les autres groupes infestés. Les premiers présentaient aussi des réductions apparentes de la croissance. Chez les témoins non infestés, la mortalité était faible (0,7–2,6%), et le degré de surpeuplement ou le débit d'eau n'avait pas d'effet significatif sur la croissance ni sur la mortalité. Les saumons infestés étaient aussi moins résistants à l'épreuve de l'eau de mer; tous les poissons maintenus à de fortes densités de peuplement ou à un faible débit d'eau sont morts dans l'eau de mer. Ces résultats indiquent que les infestations à *Ichthyobodo* ont des effets marqués à la fois sur la croissance et sur la survie de l'hôte quand ces infestations se combinent avec un stress environnemental induit par le surpeuplement et ou l'insuffisance de l'apport d'eau, bien que la densité des parasites ne soit pas affectée par ces facteurs.

[Traduit par la Rédaction]

Disease is a result of complex interactions between host, pathogen, and environment (Snieszko 1974). It is well accepted that environmental stress reduces host resistance against invading pathogens (Wedemeyer 1970; Hoffman 1976; Schreck 1981; Wedemeyer and McLeay 1981; Shepherd 1988). Hatchery practices such as high rearing density and poor water quality can affect the physiological status of anadromous salmonids (Eddy 1981; Fagerlund et al. 1981; Schreck et al. 1985; Patino et al. 1986; Boeuf and Gaignon 1989) and also their return rate (Hosmer et al. 1979). Thus it is important to define safe levels of water quality and crowding in the course of hatchery practices. However, no single aspect of water quality should be considered in isolation from the influence of other stress factors. Smart (1981) has pointed out that nutritional deficiencies and protozoan infections may reduce the ability of fish to withstand any deterioration in water quality.

The bodonid flagellate *Ichthyobodo necator* (Henneguy, 1883) is a widespread ectoparasite of various fishes and

Received May 19, 1994. Accepted February 15, 1995.
J12798

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Table 1. Fish density, dissolved oxygen (DO), and NH_4–N concentrations in tanks with different degrees of crowding and water inflow rate.

Group[a]	Fish density ($kg \cdot m^{-3}$)				DO ($mg \cdot L^{-1}$)			NH_4–N ($mg \cdot L^{-1}$)
	Week 0	Week 1	Week 3	Week 5	Weeks 0–1	Weeks 1–3	Weeks 3–5	Weeks 0–5
Infected group								
LD-MI	8.9	10.3	14.5	13.2	8.8±0.5[b]	9.0±0.2	9.0±0.2	0.005±0.001
MD-LI	17.9	21.1	18.9	3.9	6.0±0.7	7.3±1.0	9.2±0.2	0.012±0.007
MD-MI	18.2	21.3	28.2	24.9	7.8±0.4	7.8±0.3	8.0±0.3	0.014±0.001
MD-HI	17.4	23.6	28.2	21.7	9.0±0.4	8.9±0.2	9.0±0.3	0.007±0.002
HD-MI	34.7	42.8	36.9	23.6	5.7±0.5	8.0±0.8	7.5±0.4	0.028±0.002
Control group								
LD-MI	8.7	11.1	15.2	17.7	8.9±0.4	8.7±0.3	8.7±0.3	0.001±0.002
MD-LI	19.1	22.2	30.2	32.8	6.5±0.5	5.8±0.3	5.5±0.4	0.035±0.001
MD-MI	19.6	23.7	31.9	38.6	7.8±0.5	7.1±0.3	7.1±0.4	0.018±0.007
MD-HI	19.5	21.6	31.1	36.1	8.9±0.4	8.7±0.3	8.6±0.3	0.004±0.003
HD-MI	38.3	49.2	61.3	70.2	5.6±0.5	5.7±0.3	5.5±0.6	0.031±0.005

[a] LD, low rearing density (300 fish); MD, medium rearing density (600 fish); HD, high rearing density (1200 fish); LI, low water inflow (370 $mL \cdot min^{-1}$); MI, medium water inflow (750 $mL \cdot min^{-1}$); HI, high water inflow (1500 $mL \cdot min^{-1}$).
[b] Mean ± SD.

causes a serious disease in freshwater and marine aquaculture (Robertson 1985). The parasite can affect freshwater survival and seawater adaptability in young anadromous salmonids because of skin destruction, resulting in reduced early marine survival (Urawa 1992*a*, 1992*b*, 1993). Although it is generally believed that the disease occurs only under poor rearing conditions (Bauer 1959; Wedemeyer and McLeay 1981), heavy infections are frequently observed among hatchery-reared salmonids even under optimum conditions (Urawa 1992*c*). There is no experimental evidence to confirm the relationship between outbreaks of this parasitic disease and rearing conditions.

The purpose of this study was to investigate the effects of rearing density and water inflow rate on the abundance of *I. necator* and the degree of its pathogenicity to juvenile chum salmon (*Oncorhynchus keta*).

Materials and methods

Parasite-free chum salmon juveniles (mean wet weight 0.45 g, $N = 120$) were used for this experiment. A stock of 6600 fish was divided equally into two 40-L tanks, each supplied with a constant flow of 10.5°C well water. One group was kept for 1 week with 20 caged chum salmon fry infected with *I. necator* derived from Yoichi Hatchery, western Hokkaido, and the other group served as an uninfected control. Both groups were fed commercial dry pellets at about 2% of body weight per day for 1 week.

Prevalence of infection was 100% in the infected group 1 week after exposure to the infected fish. The infected group was divided into five subgroups consisting of one high density (HD, 1200 fish), three medium density (MD, 600 fish each), and one low density (LD, 300 fish) subgroup. MD subgroups were supplied with well water at three different flow rates, 370 (low inflow, LI), 750 (medium inflow, MI), or 1500 $mL \cdot min^{-1}$ (high inflow, HI). HD and LD subgroups were supplied water at MI rate. The control fish were also subdivided into the same number of subgroups as the infected group and subjected to the same conditions of stocking density and water flow. Each subgroup of infected and control fish was kept in an 18-L tank and fed as previously described for the experimental period of 5 weeks. Water temperature and dissolved oxygen (DO) concentration were monitored in each tank outlet at 2 to 3-d intervals with a DO meter (YSI model 57), and ammonia concentration was measured at about 1-week intervals using the indophenol blue method. Water temperatures were kept at 10.5°C in all tanks throughout the experiment.

Fish ($N = 20$ or 30) were sampled from each tank at 0, 1, 3, and 5 weeks after the start of the experiment. The fish were anaesthetized using MS222 (about 100 $mg \cdot L^{-1}$), immediately weighed and measured (fork length), and fixed with 10% neutral buffered formalin for parasite counts. The parasites were counted on the dorsal and ventral fins, and the body surface between these fins with a scanning electron microscope (SEM), as described by Urawa (1992*a*). Condition factor (CF) was calculated from the formula: $CF = 100w \cdot l^{-3}$, where w is the weight of each fish and l is corresponding fork length.

For the evaluation of seawater adaptability, seawater challenge tests were carried out on 30 fish from each group at week 3 in 20-L tanks supplied with aerated seawater (salinity 33‰; temperature 11°C) for 48 h. For serum chloride (Cl^-) measurements just before and after seawater challenge tests, fish were bled from the caudal vessels into heparinized hematocrit centrifuge tubes. The tubes were immediately centrifuged at 12 000 rpm for 5 min, and serum Cl^- concentrations measured by chloride counter (Hiranuma Co.).

The Mann–Whitney and Kruskall–Wallis tests (one way) were used to assess the significance of differences in parasite

Table 2. Density of *Ichthyobodo necator* on juvenile chum salmon (N = 10 each) reared in tanks with different degrees of crowding and water inflow rate for 5 weeks.

Infected group[a]	Parasite density on the skin (mm^{-2})			Parasite density on the fins (mm^{-2})		
	Week 0	Week 3	Week 5	Week 0	Week 3	Week 5
LD–MI	1.2±1.3[b]	1625±1339	3552±2167	4.3±2.5	2958±1236	6214±3167
MD–LI	3.4±2.2	1568±981	2717±1270	8.1±3.5	4135±1528	7121±1710
MD–MI	1.7±1.3	1604±1398	3110±2442	4.9±2.7	4067±1624	6423±2937
MD–HI	3.4±2.6	1021±866	4090±1975	5.5±4.5	3407±1593	7226±3940
HD–MI	1.2±0.7	570±586	3503±2318	5.4±4.2	3180±1640	5718±2311

[a] LD, low rearing density; MD, medium rearing density; HD, high rearing density; LI, low water inflow; MI, medium water inflow; HI, high water inflow.
[b] Mean ± SD.

Fig. 1. Effects of rearing density (A) and water inflow rate (B) on the mean density of *Ichthyobodo necator* on the skin and fins of juvenile chum salmon (N = 10 each). Water inflow rate and rearing density were adjusted at medium levels in groups A and B, respectively.

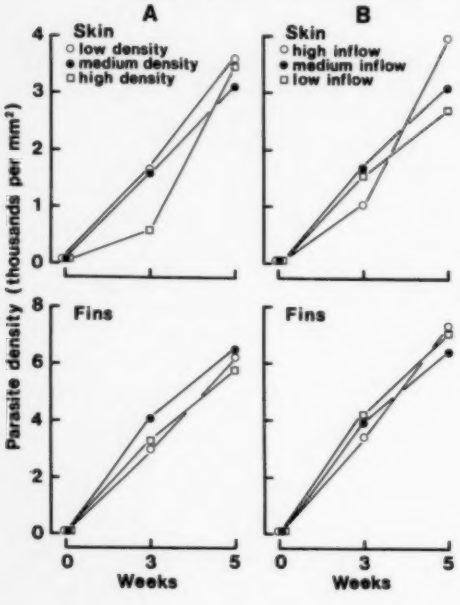
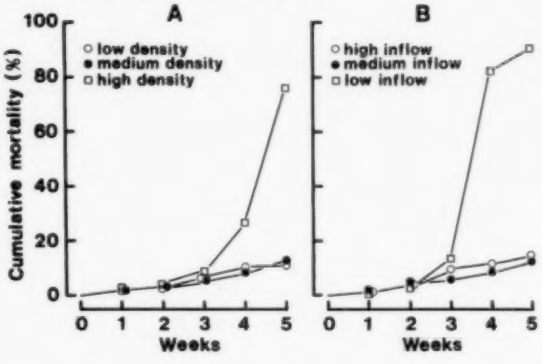


Fig. 2. Effects of rearing density (A) and water inflow rate (B) on the cumulative mortality of juvenile chum salmon infected with *Ichthyobodo necator*. Water inflow rate and rearing density were adjusted at medium levels in groups A and B, respectively.



density, body weights, CF, and serum Cl^- levels among groups. The significance level was set at $P < 0.05$ for all comparisons.

Results

Rearing densities, and dissolved oxygen and NH_4–N concentrations for the five different combinations of crowding and inflow rate throughout the experimental period of 5 weeks are presented in Table 1. Dissolved oxygen levels were reduced to lower than 7 $mg \cdot L^{-1}$ for the 1st week in the tanks of HD–MI and MD–LI in both infected and control subgroups. NH_4–N concentrations were also highest (12–35 $\mu g \cdot L^{-1}$) in these tanks, but these were within safe levels for salmonids (Smart 1981).

Initial parasite density averaged 2.2 and 6.6 parasites·mm^{-2} on the skin and fins, respectively, with no significant differences among subgroups (Table 2). By the end of the experiment (week 5), the parasite density had increased to 2700–4100 and 6200–7200 parasites·mm^{-2} on the skin and fins, respectively, with no significant differences among subgroups (Fig. 1). Control fish remained uninfected throughout the experiment.

The weekly mortality rate increased rapidly in HD–MI and MD–LI subgroups from week 3, cumulating at 76 and 90%, respectively, by the end of the experiment (Fig. 2). The total mortality was 11.4–14.5% in the other infected subgroups and only 0.7–2.6% in the controls.

The mean body weights of infected fish in the HD–MI and MD–LI subgroups were significantly lower than in the corresponding controls at week 3 (Fig. 3). Significant differences were also observed in the HD, MD, MI, and HI subgroups at week 5. The condition factor of fish in the HD and LI subgroups was significantly lower in infected fish than in the controls at weeks 3 and 5, except for the

Fig. 3. Effects of rearing density (A) and water inflow rate (B) on the growth of juvenile chum salmon. Open and shaded columns represent the uninfected control and *Ichthyobodo*-infected groups, respectively. Number of fish measured was 30 at weeks 0 and 3, and 20 at weeks 1 and 5. Scale bars represent SD. HD, high rearing density; MD, medium rearing density; LD, low rearing density; LI, low water inflow; MI, medium water inflow; HI, high water inflow; **, $P < 0.01$.

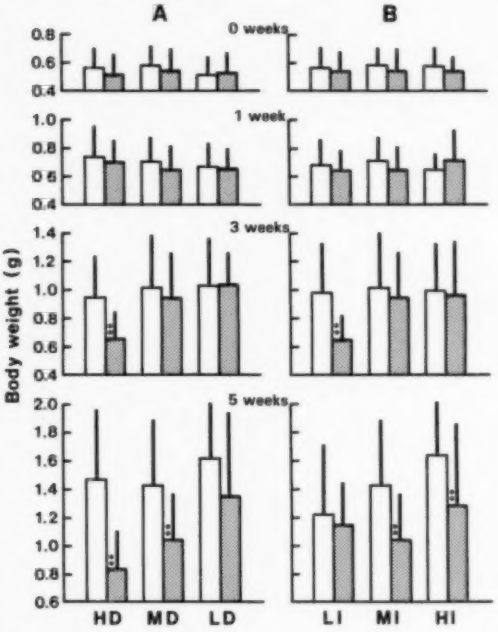
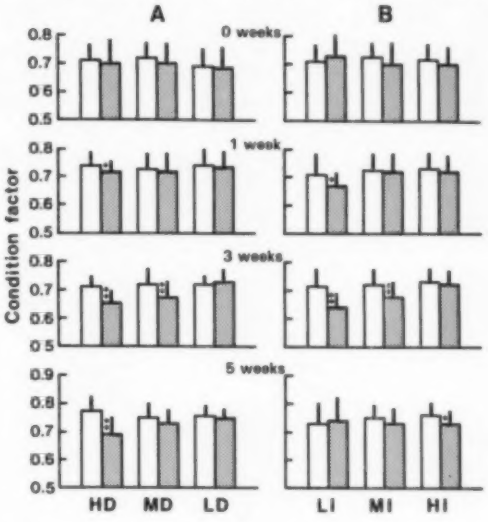


Fig. 4. Effects of rearing density (A) and water inflow rate (B) on the condition factor of juvenile chum salmon. Open and shaded columns represent the uninfected control and *Ichthyobodo*-infected groups, respectively. Number of fish measured was 30 at weeks 0 and 3, and 20 at weeks 1 and 5. Scale bars represent SD. HD, high rearing density; MD, medium rearing density; LD, low rearing density; LI, low water inflow; MI, medium water inflow; HI, high water inflow; *, $P < 0.05$; **, $P < 0.01$.



LI subgroup at week 5 (Fig. 4). Among the infected subgroups, there was a negative relation between condition factor and rearing density at weeks 3 and 5, and a positive relation between condition factor and inflow rate at week 3. In the controls, the condition factor was not influenced by fish density nor inflow rate over the experimental period.

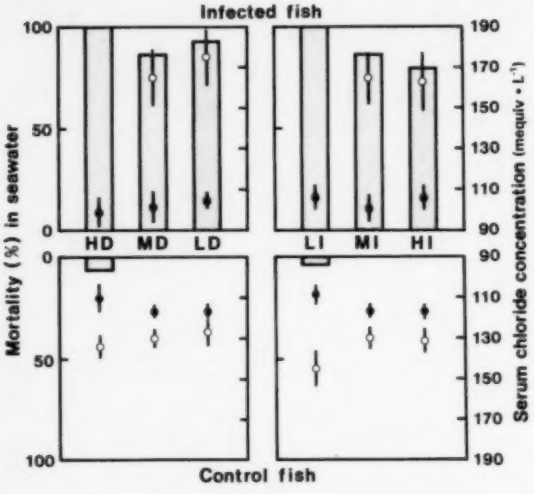
Seawater challenge tests (48 h) produced high mortalities (80–100%) in all the infected subgroups (Fig. 5). In particular, all test fish died in the infected HD and LI subgroups. The serum Cl^- concentration of infected fish was significantly higher (over 160 mequiv·L^{-1}) in seawater and lower (under 110 mequiv·L^{-1}) in fresh water compared with that of normal fish. Seawater mortalities were rare (0–6.7%) in the control subgroups, while the serum Cl^- concentrations of the HD and LI subgroups were significantly higher in seawater and lower in fresh water than those of the other control subgroups.

Discussion

This experiment reveals that the pathological potential of *Ichthyobodo necator* is strongly intensified by unfavorable rearing conditions, although the density of *I. necator* was not influenced by fish density or inflow rate. High rearing density (HD) and inadequately low inflow rate (LI) resulted in reduced survival and growth of infected fish, while these conditions did not affect the uninfected controls. The unfavorable rearing conditions induced low dissolved oxygen (5–6 mg·L^{-1}) in both the infected and control subgroups. These low dissolved oxygen levels may be more stressful to *Ichthyobodo*-infected fish than to normal uninfected fish. Itazawa (1971) suggested that the minimum level of dissolved oxygen required to maintain maximum feeding, growth, and efficiency of food conversion is 4–4.5 mg·L^{-1} for rainbow trout (*Oncorhynchus mykiss*) at about 10.5°C. Alabaster and Lloyd (1982) considered that the minimum sustained dissolved oxygen necessary for the normal life cycle of salmonids is 5 mg·L^{-1} under favorable conditions. The present study suggests that these minimum oxygen levels are dangerous for *Ichthyobodo*-infected salmonids.

Urawa (1993) reported that *Ichthyobodo* infections strongly reduce the seawater survival of juvenile chum salmon because of skin destruction. The present seawater challenge tests also showed that mass mortality (80–100%) occurred in all infected subgroups, with the highest mortality (100%) in the unfavorable rearing subgroups (HD and LI). Direct transfer to seawater may impose a considerable stress, even on healthy anadromous fish. While no significant differences in seawater mortality were detected among the controls, the serum Cl^- levels of the HD and LI subgroups were significantly lower in fresh water and higher in seawater than that of the other control subgroups. This suggests that stress induced by unfavorable rearing conditions disturbs the osmoregulation of anadromous fish,

Fig. 5. Comparison of seawater mortality (salinity 33‰ for 48 h; shaded columns), and serum Cl^- concentrations in fresh water (solid circles) and seawater (open circles) after 48 h of transfer between *Ichthyobodo*-infected juvenile chum salmon and the uninfected controls at week 3. Scale bars indicate SD. HD, high rearing density; MD, medium rearing density; LD, low rearing density; LI, low water inflow; MI, medium water inflow; HI, high water inflow.



as reported by several workers (Redding and Schreck 1983; Schreck et al. 1985; Boeuf and Gaignon 1989).

It is generally believed that crowding of fish is conducive to the development and spread of ectoparasitic diseases (Hoffman 1976). In the present experiment, however, the number of *I. necator* increased rapidly on host fish regardless of rearing conditions such as rearing density and inflow rate. This implies that *I. necator* can multiply and spread among hatchery-reared fish independent of host condition once the parasite has successfully settled on host fish. This is evidenced by the fact that heavy *Ichthyobodo* infections occur very frequently in hatchery salmonids reared even under optimal conditions (Urawa 1992*c*).

In conclusion, *Ichthyobodo* infections have marked effects on both host growth and survival, especially when the infections are combined with environmental stress induced by overcrowding or inadequate water supply, although the parasite density is not influenced by these factors.

Acknowledgments

I am grateful to Kiyokuni Muroga of Hiroshima University and J. Richard Arthur of Maurice Lamontagne Institute for their valuable comments on earlier drafts of this manuscript and to Ikutaro Shimizu of Hokkaido Salmon Hatchery for measuring ammonia concentrations.

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Origins of the Monogenea of selected major taxa of Nearctic freshwater fishes

Mary Beverley-Burton

Abstract: Comment on the origin(s) of a group of parasites requires an awareness of the paleogeology of the region and historical records – phylogenies of both hosts and their parasites. The paleogeology of the Nearctic Region is described briefly and 11 families of Nearctic freshwater fishes for which phylogenetic information is available are considered: Acipenseridae, Polyodontidae, Catostomidae, Ictaluridae, Fundulidae, Poeciliidae, Cyprinodontidae, Percidae, Centrarchidae, Moronidae, and Percopsidae. A previous hypothesis that the Nearctic ancyrocephalids are of mixed origins is supported and the phenomena of vicariance, dispersal, host-switching, and co-evolution are demonstrated.

Résumé : Pour faire des observations sur l'origine d'un groupe de parasites, il faut connaître la paléogéologie de la région et avoir des données historiques–phylogénétiques sur les hôtes et leurs parasites. Nous décrivons brièvement la paléogéologie de la région néarctique et examinons onze familles de poissons d'eau douce néarctiques pour lesquels il existe de l'information phylogénétique : Acipenseridae, Polyodontidae, Catostomidae, Ictaluridae, Fundulidae,Poeciliidae, Cyprinodontidae, Percidae, Centrarchidae, Moronidae et Percopsidae. Nous confirmons l'hypothèse selon laquelle les ancyrocéphalides seraient d'origines diverses, et démontrons les phénomènes de vicariance, de dispersion, de changement d'hôte et de co-évolution.

[Traduit par la Rédaction]

Introduction

The origin(s) of the ancyrocephalid Monogenea parasitizing the freshwater fishes of the Nearctic drew opposing speculative comment from Gusev (1978), who considered they were distinct and Cone (1978) who suggested they were of mixed origins. At that time the systematics of the North American ancyrocephalids (sensu Beverley-Burton 1984) was in a chaotic state and knowledge of other major monogenean taxa was somewhat fragmentary.

To present hypotheses on the origins of parasitic animals and their hosts, the following information is required: a paleogeologic history of the region; a historical record–phylogeny of the host taxon; and a historical record – phylogeny of the parasites. It is only in rare situations that all these requirements are met. In the present case, the paleogeology of the Nearctic has become increasingly well understood in the last 20 years; phylogenetic analyses and commentary on several Nearctic piscine taxa became available with the publication of Mayden (1992); and several groups of Nearctic Monogenea have been the subject of systematic review (including some phylogenetic analyses) by Beverley-Burton and her co-workers since 1981 (see Beverley-Burton and Suriano 1981; Suriano and Beverley-Burton 1981, 1982; Klassen and Beverley-Burton 1985, 1987, 1988*a*, 1988*b*; Klassen et al. 1985; Murith and Beverley-Burton 1985; Beverley-Burton et al. 1986, 1991; Wheeler and Beverley-Burton 1989*a*, 1989*b*; Beverley-Burton and Klassen 1990; Chisholm et al. 1991).

The purpose of the present paper is to draw together and review the available information on carefully selected North American fish taxa and their Monogenea in an attempt to provide a meaningful language or Manter's "parascript" for these parasites that "tells of themselves and their hosts both of today and yesteryear" (see Brooks and McLennan 1993). Although I recognize that there are many problematic areas and gaps, the examples I have chosen illustrate the effect of vicariance, dispersal, host-switching, and co-evolution and allow putative conclusions on the origins of these parasites.

Paleogeology of the Nearctic Region: historical aspects

To appreciate the present-day distribution of the Nearctic freshwater fishes and their monogenean parasites, it is necessary to have an awareness of the paleogeology of the world, especially the vicariant events from the Upper Permian Period, through the Mesozoic Era (Triassic, Jurassic, and Cretaceous) to the end of the Tertiary (geologic time nomenclature from Dodson and Dodson 1985). Both Cox (1974) and Howarth (1981) provide a general global backdrop to the paleogeology of the major land masses. In particular, Howarth considers the paleogeology of the north polar regions up to the end of the Cretaceous. Matthews (1979), as a preliminary to his analyses of the

Received March 23, 1994. Accepted February 15, 1995. J12779

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Canadian insect fauna, outlined a historical background that included putative land routes across the North Atlantic Ocean (the De Geer, Greenland – Faeroes, and Belle Isle – Rockall bridges) together with comment on climatic variations in the context of animal migrations. In addition, Matthews (1979) presented maps showing putative land connections in the Beringial region that may have enhanced the possibility of dispersals (both faunal and floral) to and from Asia. Similarly, Noonan (1988) gave an account of past land configurations and climate in the Mesozoic as a preliminary to his hypotheses on the faunal relationships of insects between the eastern Nearctic and Europe. Frakes (1979) noted the mild climate of the Mesozoic Era, including the great warmth at high latitudes of the Cretaceous, which may have facilitated dispersals.

It was not until the Upper Pliocene Epoch that a continuous land route, the isthmus of Panama, was established between North and South America (Donnelly 1988) bringing together previous components of Laurasia and Gondwanaland.

Terminology

In addition to an awareness of the paleogeologic changes that have shaped the configuration and isolation of the present-day Nearctic, it is necessary to appreciate the following terms that refer to the phenomena, both geographic and ecologic, that have impacted on the evolution and extant distributions of the Nearctic Monogenea and their hosts. "Vicariance" refers to a geographic separation of a continuous biota (Wiley 1981); examples of geographic barriers include continental break-up (following movement of tectonic plates), climatic changes (desertification or glaciation), orogeny (emergence of mountain ranges), marine incursions (inundations of the sea), and shifting drainage patterns. "Dispersal" is the transport or movement of organisms into geographic regions away from their normal range, which may include their site of origin (Krebs 1994). "Coevolution" indicates situations where taxa of different phylogenetic affinities (e.g., hosts and their parasites) evolve together, i.e., Fahrenholt's rule that "parasite phylogenies mirror host phylogenies" (see Brooks 1979). "Host-switching" (see Brooks 1988) refers to a changing ecological association when parasites (usually the larval stage, in the present case the oncomiracidia) transfer to a different host taxon.

The taxonomic affinities and authors of the monogenean genera and suprageneric taxa are as given by Beverley-Burton (1984).

The Nearctic Ichthyofauna

The Nearctic Region has been regarded by Cox and Moore (1980) as a faunistic island with oceanic barriers virtually separating the Americas from each other and completely isolating them from other continents.

Although biotic exchange has occurred between the Nearctic and Neotropical Regions, the freshwater ichthyofauna has not been greatly involved (see Burr and Mayden 1992): there are no riverine north–south corridors and considerable mountain and climatic barriers exist. The only families of Neotropical fishes that are found in the Nearctic are poorly represented and restricted to the south of the Region, e.g., cichlids, characids, and poeciliids (Burr and Mayden 1992). Thus, in the present context, the island concept of Cox and Moore (1980) is valid. Burr and Mayden (1992) recognized 18 faunal provinces in North America and noted that there are major variations in diversity of freshwater fish species over the Nearctic as a whole: Canada and Alaska comprise more than 50% of the land mass but have relatively few species (none of which are enzootic) while the Mississippi province has 375 species (34% of which are enzootic). In addition, the eastern and western ichthyofaunas are distinctive: the majority of the 1061 Nearctic species are found in river drainages east of the Rockies, while only about 300 species occur in the west. Gilbert (1976) listed Eurasia, Central America, South America, and the marine environment as sites of origin for Nearctic freshwater fishes and also recognized enzootic taxa. Putative origins for the taxa considered in the present paper are included in Table 1. Cox and Moore (1980) commented on the faunistic similarity of the Palaearctic and the Nearctic regions because of their climate and the fact that animals have been able to migrate from one to the other intermittently through geologic time.

At least 1061 species of 201 genera have been recorded from the fresh waters of North America (Burr and Mayden 1992) and, of the 50 families listed, 8 are considered to be enzootic. Burr and Mayden (1992) follow Moyle and Cech (1988) in the recognition of two types of fishes in fresh water: euryhaline, marine fishes, and obligatory freshwater fishes. In the latter group, Burr and Mayden (1992) list 30 families as freshwater dispersants, incapable of travelling long distances through salt water, and 20 families of saltwater dispersants. Berra (1981) regarded the following 10 families that occur in the Nearctic as being "mostly marine": Anguillidae, Engraulidae, Ariidae, Atherinidae, Hemirhamphidae, Belonidae, Syngnathidae, Mugilidae, Gobiidae, and Soleidae, and these are omitted from further consideration in the present paper. Twenty-nine other families are excluded for a variety of reasons, e.g., they are not known to be parasitized by Monogenea; they are represented in fresh water by only a few species; they are of limited distribution at the southern edge of the Nearctic Region; or phylogenetic information is lacking.

It is noteworthy that members of some families of Nearctic freshwater fishes are often hosts to infestations of Monogenea, e.g., the Cyprinidae, which carry dactylogyrids (primarily *Dactylogyrus* spp.) and gyrodactylids (primarily *Gyrodactylus* spp.), and the Salmonidae, which carry gyrodactylids (primarily *Gyrodactylus* spp.). However, in both cases there is a lack of phylogenetic reconstruction for the parasites and (or) their hosts, so that it is impossible to put forward any hypotheses on the origin of their parasites at this time.

The 11 families of fishes that are considered in the present paper are listed in Table 1.

Origins of the Monogenea of selected freshwater Nearctic fishes

Acepenseriformes: Acipenseridae and Polyodontidae (sturgeons and paddlefish)

Burr and Mayden (1992) have briefly outlined the confusion

Table 1. Families of Nearctic freshwater fishes in the context of the origin of their monogenean parasites.

Family[a]	No. of species in fresh water[a]	Taxonomic affinity[b]	Putative origin[c]	Major monogenean taxon/taxa considered
Acipenseridae	8	Acipenseriformes	Mixed	Diclybothriidae: *Diclybothrium* and *Paradiclybothrium*
Polyodontidae	1	Acipenseriformes	Mixed	Diclybothriidae: *Diclybothrium*
Catostomidae	70	Cyprinoformes	Enzootic	Dactylogyridae: *Pellucidhaptor* Pseudomurraytrematidae
Ictaluridae	48	Siluriformes	Enzootic	Ancyrocephalidae: *Ligictalurus*
Fundulidae[d]	38	Atheriniformes	Marine	Ancyrocephalidae: *Salsuginus*
Poeciliidae	75	Atheriniformes	Central America	Ancyrocephalidae: *Salsuginus*
Cyprinodontidae	30	Atheriniformes	Marine	Ancyrocephalidae: *Salsuginus*
Percidae	165	Perciformes	Eurasia	Ancyrocephalidae: *Urocleidus* and *Aethycteron*
Centrarchidae	32	Perciformes	Enzootic	Ancyrocephalidae: *Onchocleidus* and "articulating bar group"[e]
Moronidae	4	Perciformes	Enzootic	Ancyrocephalidae: *Onchocleidus* Microcotylidae: *Pauciconfibula*
Percopsidae	2	Percopsiformes	Enzootic	Ancyrocephalidae: *Urocleidus*

[a]Familial nomenclature and number of species from Burr and Mayden (1992).
[b]Taxonomic affinities based on Berra (1981).
[c]Putative origin based on Gilbert (1976).
[d]Including the Profundulidae of Burr and Mayden (1992).
[e]See Beverley-Burton and Klassen (1990).

regarding the phylogenetic relationships of the Acipenseridae and the Polyodontidae within the Acipenseriformes. Birstein (1993) supposed the "chondrosteans" originated, in the early Triassic, in the fresh waters of northern Asia. Bemis et al. (1994) recognized the antiquity of the Acipenseriformes, which go back to the Jurassic, and suggested from fossil evidence that the group has always had a Holarctic distribution. Birstein (1993) believed the sturgeons (Acipenseridae) became widespread in the northern hemisphere in the late Cretaceous before the divergence of the paddlefishes (Polyodontidae). Gardiner (1984) reported that the earliest known fossil remains of species of *Acipenser* occur in Alberta and are from the upper Cretaceous, while later fossils of *Acipenser* spp. are known from more widespread areas in the Nearctic and, throughout the Tertiary, are recorded from Europe as well as North America.

Bemis et al. (1994) list four fundamental features pertaining to the biology of the Acipenseriformes, which include anadromy and dermersal spawning. Primarily, sturgeons are gravel spawners in riverine situations, with the resultant offspring moving into salt water to feed for extended periods of time and only returning to fresh water as breeding adults (E. Balon, University of Guelph, personal communication). Thus, Nearctic species that are considered to be "fresh water" (e.g., *A. fulvescens*) or "freshwater amphidromous" (e.g., *A. brevirostrum*) (see Kieffer and Kynard 1993) are secondarily adapted from anadromous ancestors.

The hypothesis that the sturgeons (*Acipenser* spp.) represent a Holarctic group and that those now inhabiting North America may be descended from an ancestral lineage(s) that became separated by vicariant events involving the formation of the Atlantic, with the tectonic movement of the Nearctic away from the Palearctic, is supported by the distribution of the "Atlantic sturgeon": *A. oxyrhynchus* of the western Atlantic may be conspecific with *A. sturio* of the eastern Atlantic, which re-enforces the idea of the separation of the two "stocks" by a vicariant event (E. Balon, personal communication). Rochard et al. (1990) listed 17 extant *Acipenser* spp., of which five occur in North America: *A. flavescens*, *A. brevirostrum*, *A. oxyrhynchus*, *A. transmontanus*, and *A. medirostris*.

The monogenean *Diclybothrium armatum* Leukart, 1835 is found on the gills of both *A. fulvescens* and *A. brevirostrum* (see Beverley-Burton 1984). Rochard et al. (1990) noted that of the five Nearctic sturgeons, *A. fulvescens* is a "freshwater" species and *A. brevirostrum* has some populations occurring in fresh water and others that utilize estuarine and marine locations. Bain (1994) considered *A. brevirostrum* to be a "potanadromous" or "facultative anadromous" species, as the fish has rarely been taken outside the coastal influence of it's home rivers. Both species are enzootic to the Nearctic Region. MacNeill and Busch (1994) reported the belief that lake sturgeon (*A. fulvescens*) evolved in the early Mississippi river drainage and entered the Great Lakes region in the wake of the receding glaciers 10 000 to 14 000 years ago. In Europe, *D. armatum* occurs on *A. baeri* and *A. ruthenus*, which are "predominantly" fresh water (see Choudhury and Dick 1993).

Choudhury and Dick (1993) surveyed the parasites of lake sturgeon from central Canada, finding *D. armatum* in 70% of 218 fish and therefore regarding it as a "core" species and a parasite of Acipenseriformes in general. Because there was a low index of similarity (8%) between parasites of *A. fulvescens* and other North American sturgeons, which are anadromous, in contrast with a higher

index (28%) when compared with the freshwater sturgeons of Eurasia, Choudhury and Dick (1993) suggested that a freshwater life history was of greater importance than continental isolation from other freshwater sturgeons in the determination of the parasite fauna of these fishes.

It is hypothesized that, sometime in the Tertiary, *Diclybothrium armatum* parasitized freshwater sturgeons across a wide geographic range. When the Nearctic and Palearctic Regions separated, two lineages of the parasite occurred: the Nearctic lineage on the sturgeons which gave rise to *A. fulvescens*, and the Palearctic lineage on the freshwater sturgeons of Eurasia. In the Nearctic Region, the parasite might have transferred to a population of *A. brevirostrum* that was tied to a freshwater life cycle.

A second species of *Diclybothrium*, *D. hammulatum* (Simer, 1929), has been reported in North America from *A. fulvescens* (see Beverley-Burton 1984) and *Polyodon spathula* (see Hoffman 1967). The paddlefish genus *Polyodon* is considered to be enzootic in the Nearctic Region (Burr and Mayden 1992) but, as neither *Polyodon* nor *Psephurus* from eastern China are known from the fossil record (Gardiner 1984) the place of origin of the *Polyodontidae* cannot be determined. However, if we assume that paddlefish occurred in the Mississippi basin, together with the evolving *Acipenser* spp., which were carrying a diclybothriid, it is postulated that, by host-switching, it acquired the parasite that subsequently evolved as *D. hammulatum* and that today parasitizes both *A. flavescens* and *P. spathula*.

It is of interest that neither *A. transmontanus* of western North America nor *Scaphirhynchus* spp., primarily of the Mississippi river drainage (Lee et al. 1980), are parasitized by diclybothriids. Although the ranges of *Scaphirhynchus albus* and *S. platyrhynchus* coincide to a large extent with that of *A. fulvescens*, it appears host-switching has not occurred.

Acipenser medirostris, which is found along the Pacific coast of both Asia and North America (Rochard et al. 1990), is parasitized by *Paradiclybothrium pacificum* Bykhovsky and Gusev, 1950 in Asia. However, this parasite has not been reported from North America. This may be due to the disjunct populations of *A. medirostris*. The origin of *Paradiclybothrium* is unknown, but if one assumes that the ancestor of *Diclybothrium* and *Paradiclybothrium* occurred on acipenserids during the Cretaceous and early Tertiary, it is possible that *Paradiclybothrium* developed in the east, while *Diclybothrium* developed westwards. The two lineages may have been isolated by a marine incursion, such as the Tethys Sea. Anthony (1974) reported a diclybothriid from *A. fulvescens* taken in Ontario, which was tentatively identified as *Paradiclybothrium* sp. No details of the material were published and specimens are unavailable. It seems highly unlikely that the genus is represented in Ontario.

Catostomidae (suckers)

Catostomids occur in temperate lakes and rivers in the Nearctic and eastern Asia (Smith 1992). The family is thought to have evolved from large, deep-bodied fishes, inhabiting low gradient waters, towards more slender, smaller fishes living in medium to small tributaries of

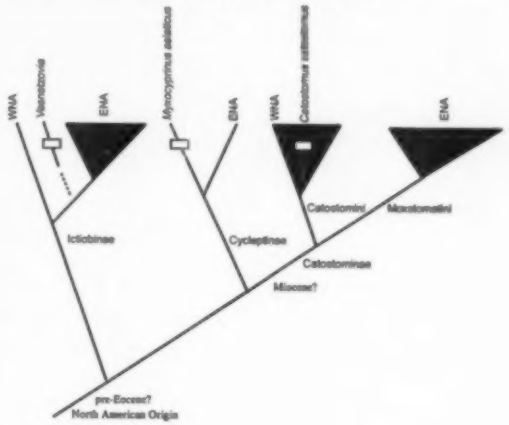

Fig. 1. Reduced area cladogram of catostomids (suckers) based on Smith (1992) with superimposition of geographic distribution. Triangles represent multiple species (ENA, eastern Nearctic; WNA, western Nearctic). White oblongs represent independent invasions of the eastern Palearctic (*Vasnetzovia* in eastern Siberia; *Myxocyprinus asiaticus* in China; *Catostomus catostomus* in eastern Siberia, this species is also widely dispersed in eastern North America (Lee et al. 1980)).

intermediate gradient. Smith (1992) presented a phylogeny for the family and recognized three subfamilies: the Ictiobinae and Cycleptinae, which together comprise 10 species assigned to the morphologically primitive genera inhabiting larger bodies of water, and the Catostominae, with a comparatively large number of species that are slender and occur in rivers and streams.

There has been considerable controversy concerning the derivation of the catostomids from an ancestral group and their site of origin: the two major hypotheses concerning the origin of the Catostomidae are that the family originated in Asia with subsequent dispersal into North America or that the family originated in the Nearctic with dispersal to Asia (see Berra 1981). Although Smith (1992) discusses the evolution of the family, he does not address the problem of origin.

It might be argued that, since ictiobines (fossil) and cycleptines (extant *Myxocyprinus*) are present in Asia, the cladogram offered by Smith (1992) could support the hypothesis that the Catostomidae is of Asiamerican origin with no need for dispersal (except perhaps for *Catostomus*). However, by superimposing the geographic distributions of catostomids on Smith's cladogram (Fig. 1) it is seen that the most primitive catostomids, the Ictiobinae (represented by the fossil *Amyzon*), occur in western North America and that there are few catostomids occurring outside of the Nearctic. This strongly suggests that catostomids originated in North America and that the dispersal of the ictiobine *Vasnetzovia*, the cycleptine *Myxocyprinus asiaticus*, and the catostomine *Catostomus catostomus* to Asia occurred via a Bering land bridge, in three independent occurrences. Therefore, in the present context, the Catostomidae is regarded as enzootic.

Present knowledge of the 23 named species of the dactylogyrid genus *Pellucidhaptor* is inadequate to provide characters for cladistic analysis and the following comments must be recognized as being speculative, pending further research.

The majority of *Pellucidhaptor* spp. parasitize Nearctic catostomids (Beverley-Burton et al. 1991). However, cyprinids in both the Palearctic (where 4 *Pellucidhaptor* spp. have been found on the "primitive" genera *Phoxinus*, *Leuciscus*, and *Abramis* (see Gusev and Lukjanceva 1971; Gusev and Strijak 1972)) and the Nearctic (where *P. pellucidhaptor* Price and Mizelle, 1964 occurs on *Richardsonius* sp.) are also parasitized. From the cladogram presented by Coburn and Cavender (1992), *Richardsonius* clearly belongs to a widespread North American group of cyprinids (the shiners) with no obvious Eurasian connection. The presence of *Pellucidhaptor* on this host has three possible explanations: that it switched hosts from a catostomid to *Richardsonius*; that it represents a relict cyprinid parasite in North America, following the extinction of lineages on other cyprinids of the Nearctic; or that it evolved on *Richardsonius* and switched to catostomids.

Gusev and Lukjanceva (1971) assumed *Pellucidhaptor* was of Eurasian origin and put forward two hypotheses to explain it's presence in North America: that entry was with a "protocyprinid" ancestor that later evolved into the catostomid lineage with the coevolution of the *Pellucidhaptor* spp.; or that *Pellucidhaptor* entered with the minnows and oncomiracidia, unable to locate the relatively few cyprinids present in the Nearctic, switched to the well-established catostomids and subsequently diverged. As Beverley-Burton et al. (1991) pointed out, the hypotheses put forward by Gusev and Lukjanceva (1971) are unsupported and, in spite of the putative phylogenetic relationships of the North American Cyprinidae put forward by Cavender and Coburn (1992), it is still not possible to evaluate the suggested Eurasian origin of *Pellucidhaptor* because of a lack of a phylogenetic analysis for *Pellucidhaptor* species. It is timely to re-examine any available material of described *Pellucidhaptor* spp. and to extend the collection of species from targeted catostomids (e.g., *Cycleptus elongatus*, *Chamistes* spp., *Deltistes luxatus*, *Xyrauchen texanus*, *Erimyzon* spp., *Scartomyzon* spp., and *Thobumia* spp.) which have not yet been recorded as hosts for this group of parasites.

Regardless of the origin of *Pellucidhaptor*, it is evident that it has undergone considerable radiation on the suckers in North America (Beverley-Burton et al. 1991) without evolving into distinct genera, although the hosts have done so. Beverley-Burton et al. (1991) noted that *Pellucidhaptor* has already been found on 6 genera (of 13, see Mayden and Burr 1992) and 12 species (of some 60, see Lee et al. 1980), that 9 of the 19 Nearctic *Pellucidhaptor* species parasitize more than one host species, and frequently, more than one species of *Pellucidhaptor* occur on a single host. Thus, at least in North America, *Pellucidhaptor* spp. do not show a high degree of host specificity.

A similar conundrum exists with regard to the origin of the monogenean family Pseudomurraytrematidae, which comprises four genera (*Myzotrema*, *Pseudomurraytrema*, *Anonchohaptor*, and *Icelanonchohaptor*) with 15 species of which all, except 1 (*P. asiaticus* Chang and Ji, 1978), are Nearctic, and all parasitize catostomids. As stated above, there is evidence to support the hypothesis that the Catostomidae originated within the Nearctic. If coevolution has occurred, this leads to the conclusion that the pseudomurraytrematids also have a North American origin and that *P. asiaticus*, which is found on the cycleptine *Myxocyprinus asiaticus* in China, dispersed into Asia with an ancestral host from the Nearctic across a Bering land bridge. This conclusion is supported by the putative phylogeny of the pseudomurraytrematid genera utilizing the character evolution sequence of the haptoral sclerites: the two pairs of hamuli and two transverse bars of *Myzotrema* are considered plesiomorphic, while the single pair of hamuli and three pairs of transverse bars of *Pseudomurraytrema* and the absence of hamuli and transverse haptoral bars of *Anonchohaptor* and *Icelanonchohaptor* are derived states (D.C. Kritsky, Idaho State University, personal communication). *Myzotrema* occurs on a cycleptine (which is regarded by Smith (1992) as an early lineage in catostomid evolution) in North America and is probably the first to have diverged in the pseudomurraytrematid clade (D.C. Kritsky, personal communication). Therefore it is possible the ancestor of the family occurred in the Nearctic on a primitive catostomid.

Ictaluridae (catfishes)

It is generally accepted that the ictalurids are enzootic to North America and form a monophyletic group. It seems probable they are more closely related to Asian and African siluriforms than any others (Lundberg 1992). According to Burr and Mayden (1992), the family comprises seven genera with 48 extant species. Fish from three ictalurid genera are hosts for the six known species of the ancyrocephalid genus *Ligictaluridus* (see Klassen and Beverley-Burton 1985; Klassen et al. 1985).

Klassen and Beverley-Burton (1987) presented a cladistic analysis for five *Ligictaluridus* species. The resultant cladogram did not perfectly match either of the two host cladograms discussed by Lundberg (1970), which illustrated competing hypotheses on the phylogenetic relationships of their hosts *Amiurus* (bull heads), *Schilbeodes* (madtoms), *Noturus* (stone cats), and *Ictalurus* (channel cats).

Klassen and Beverley-Burton (1987) suggested that coevolution between host and parasite may explain two-thirds of the observed host–parasite relationships and went on to consider the present-day ecology of the hosts in order to provide an explanation for the remaining host–parasite relationships: bull heads and madtoms prefer a shallow-water habitat with a muddy bottom and rather dense vegetation; stone cats are found in rapidly flowing deep streams and rivers with rocky bottoms; and channel cats are predominantly pelagic in deep rivers and lakes. It was hypothesized that an ancestral form of *Ligictaluridus* transferred by host-switching from a centrarchid to an ictalurid. This hypothesis re-enforces a general idea expressed by Gusev (1978), regarding the origin of the ancyrocephalids parasitizing Nearctic catfishes. Klassen and Beverley-Burton (1987) went on to suggest the ancyrocephalids found on *Ictalurus* (*Ictalurus*) and *Noturus* (*Noturus*) underwent allopatric speciation as their hosts

established themselves in new habitats and that the interspecific phylogeny of *Ligictaluridus* reflects adaptations to new environments rather than host taxonomy.

Fundulidae, Poeciliidae, and Cyprinodontidae (top minnows, live bearers, and pupfishes)

The ancyrocephalid genus *Salsuginus* is a monophyletic group (Beverley-Burton and Klassen 1990). The genus comprises nine described species of which seven are parasitic on *Fundulus* spp. (six in North America and one in Bermuda), one on *Gambusia affinis* from the southern United States and the Bahamas, and one on *Cyprinodon* in the Bahamas. As a generalization, the above three families (sensu Burr and Mayden 1992) all contain species that have some tolerance to salt water and, perhaps, the ability to cross saltwater barriers such as that between the North American land mass and the West Indies, the Bahamas, and Bermuda.

Cashner et al. (1992) provided a comprehensive account of the systematic controversies involving the atheriniform–cyprinodontiform fishes and noted the hypotheses put forward by Rosen (1978) and Parenti (1981). Rosen (1978) included the flying fishes and half beaks (Exocoetidae), needlefishes (Belonidae), and sauries (Scomberesocidae) in the Exocoetoidei, with *Fundulus*, *Gambusia*, and *Cyprinodon* belonging to the Cyprinodontoidei. Both Rosen (1978) and Parenti (1981) agreed that these suborders have a phylogenetic relationship that presumes a common marine ancestor.

Murith and Beverley-Burton (1985) recognized five *Salsuginus* spp. parasitizing killifish that were differentiated on slight, but constant, morphological and morphometric differences in the sclerotized components of the male copulatory apparatus and hamuli. Because of the declared phylogenetic relationships of *Fundulus* noted above, Murith and Beverley-Burton (1985) examined deposited material and (or) published descriptions of the ancyrocephalids from exocoetids, belonids, and atherinids and noted the basic similarity of the male copulatory apparatus of all these Monogenea to that of *Salsuginus*. Murith and Beverley-Burton (1985) therefore presented an hypothesis that an ancestral ancyrocephalid with this characteristic copulatory mechanism may have evolved on a marine atheriniform and given rise to species of this taxon on marine fishes, some of which moved into brackish–fresh water in association with fundulid, poeciliid, and cyprinodontid hosts. This hypothesis is in direct contrast with the implication raised by Beverley-Burton and Klassen (1990) that *Salsuginus*, because of its monophyletic status within the Nearctic freshwater ancyrocephalids, is of freshwater origin. Bearing in mind the phylogenetic relationships of *Fundulus* with marine taxa and the distribution of killifishes indicating a physiological tolerance to salt water, it seems that the question of the origin of *Salsuginus* is unresolved.

It appears that the killifishes are a group that shows little morphologic–morphometric differentiation and has evolved comparatively recently (Parenti 1981). According to Scott and Crossman (1973), killifishes are most speciose in the southeastern United States. It seems probable that the known *Salsuginus* spp. are highly host specific and may represent a phylogenetic lineage in which the morphology is, at present, somewhat uniform.

Percidae (perches and darters) and Percopsidae (trout perches)

Wiley (1992) considers that both the Percidae and Etheostomatinae are Laurasian. The sister-group relationship between the European enzootic genera *Zingel* and *Romanichthys* and the Nearctic darters (*Crystallaria*, *Percina*, and *Etheostoma*) suggests an early Tertiary origin for this clade before the final separation of the Nearctic. Thus, the darters were present in the eastern Nearctic by the Eocene. Wiley (1992) makes no comment on the origin and distribution of *Stizostedion*: although more closely related to the above clade than to *Gymnocephalus* (the third Eurasian genus of the old world darters), there is no suggestion that *Stizostedion* moved into the Nearctic in the early Tertiary. Collette and Bănărescu (1977) had suggested that percid genera had moved eastwards from Europe into Siberia during the Oligocene and then *Perca* and *Stizostedion* dispersed from Siberia into North America via a North Pacific route in the late Tertiary. Wiley's (1992) phylogenetic analysis did not support the taxonomy proposed by Collette and Bănărescu (1977) and the mechanism of entry of the large percids into the Nearctic remained unresolved. However, the fact that *Stizostedion* is nested among exclusively Eurasian genera in Wiley's phylogeny, suggests a Eurasian origin with subsequent dispersal to the Nearctic.

It is of interest that Miller (1965) commented, based on fossil evidence that, the "larger percids" should be included among the older Nearctic fauna of at least the Eocene.

The Percopsidae is considered to be enzootic to the Nearctic, with a fossil record going back to the Eocene (Burr and Mayden 1992). Although not phylogenetically related to the percids, the two families are considered together in the present paper as they are hosts to species of the same ancyrocephalid genus *Urocleidus* (sensu Beverley-Burton 1984), with three species: *U. aculeatus* (van Cleave and Mueller, 1932) from *Stizostedion* spp.; *U. adspectus* Mueller, 1936 from *Perca flavescens*; and *U. baldwini* (Dechtiar, 1974) from *Percopsis omiscomaycus*. Beverley-Burton and Klassen (1990) drew attention to the unresolved "Group B" of their phylogenetic analysis of 103 species of Nearctic ancyrocephalids. Group B comprised six species, all of which had been assigned to *Urocleidus*, but which were declared by Wheeler and Beverley-Burton (1989*a*) to be incertae sedis.

The origin of *Urocleidus* on the "larger percids" *Perca* and *Stizostedion* was considered previously by Cone (1978) and Suriano and Beverley-Burton (1981), who based their remarks on the hypothesis of Collette and Bănărescu (1977) and suggested that *Urocleidus* had been brought into the Nearctic by its percid hosts. The parasites were thought to have transferred to troutfish, *Percopsis*, by close ecological association and host-switching from *Perca* or *Stizostedion*.

A hypothesis put forward by Wheeler and Beverley-Burton (1989*b*) was that a Eurasian percid ancestor was host to a *Urocleidus* ancestor: the percids (darters), which dispersed into the Nearctic may have retained a lineage of Eurasian *Urocleidus*, which gave rise to the genus

Aethycteron (see below), while a more conservative *Urocleidus* lineage remained associated with Eurasian percids and transported into the western Nearctic as described above.

In discussing the origins of these fishes and their ancyrocephalid parasites, it is of interest to note that there are no *Stizostedion* spp. in eastern Asia (Collette and Bănărescu 1977) and, although there are five species of *Stizostedion* (three Palearctic and two Nearctic) and three species of *Perca* (all Palearctic, with *P. flavescens* being Holarctic), there are only two species of *Urocleidus* parasitizing percids and both are Nearctic (*U. aculeatus* on *S. vitreum* and *S. canadense* and *U. adspectus* on *P. flavescens*). Thus, unless there have been other *Urocleidus* spp. which became extinct, parasite speciation appears to be lagging behind host speciation. Finally, there is no evidence that *Urocleidus* spp. occur on Palearctic percids.

The darters of North America, *Crystallaria*, *Percina*, and *Etheostoma* (see Burr and Mayden 1992), comprise more than 140 species. These small, stream-loving, colourful fishes are parasitized by species of the ancyrocephalid genus *Aethycteron*, which was accepted as a monophyletic clade by Beverley-Burton and Klassen (1990). Initially, Suriano and Beverley-Burton (1982) suggested that *Aethycteron* had descended from an *Onchocleidus*-like ancestor that spread from centrarchids to darters by ecological association and host-switching. However, on closer examination of the male copulatory organ in *Aethycteron* spp., Wheeler and Beverley-Burton (1989*b*) put forward an alternative hypothesis that the ancestor of *Aethycteron* possessed an accessory piece similar to that of *Urocleidus* and thus, *Aethycteron* was derived from an ancestral *Urocleidus*. The phylogenetic evidence to support this claim was provided by Beverley-Burton and Klassen (1990). In addition, it is noteworthy that all the known species of *Urocleidus* (*sensu* Beverley-Burton 1984) and *Aethycteron* show a marked specificity for the Percidae.

Moronidae (temperate basses)

The family Moronidae is enzootic to North America and is represented by only four species (Burr and Mayden 1992). The family is monophyletic with no evidence of common ancestry with the Percichthyidae (in which it was formerly placed) or with any other percoid group (Johnson 1984). The site of origin of the moronids is controversial and the previously suggested derivation from marine serranids by Branson and Moore (1962) is no longer considered valid.

According to Lee et al. (1980) two species, *Morone americana* and *M. saxatilis*, occur in brackish water localities along the Atlantic coast and have the ability to move into fresh water, especially during spawning migrations. In contrast, *M. chrysops* and *M. mississippiensis* occur in river systems.

Temperate basses are parasitized by four species of the ancyrocephalid genus *Onchocleidus*. It was assumed by Beverley-Burton et al. (1986) that this group formed a monophyletic clade descended from a single ancestor that, through ecological association, had switched from centrarchids to moronids in or near the Mississippi drainage. However, a cladistic analysis of the 24 valid species of *Onchocleidus* by Wheeler and Beverley-Burton (1989*b*) showed that the species parasitizing the temperate basses do not form a monophyletic clade and their occurrence on the four *Morone* spp. is a result of four separate instances of host-switching, through ecological association with centrarchids.

Recently, a microcotylid, *Pauciconfibula subsolana* Chisholm, Beverley-Burton and McAlpine, 1991, has been described from *Morone americana*, collected in fresh water, in New Brunswick (Chisholm et al. 1991).

While most microcotylids are found on marine perciform fishes, in North America the freshwater drum, *Aplodinotus grunniens* (Sciaenidae), and striped bass, *M. saxatilis*, carry members of the superfamily. It may be that the freshwater drum is derived from a marine sciaenid ancestor. Further, *M. saxatilis*, which spawns in fresh water, is known to occur in salt water. Thus, I speculate that all known Nearctic microcotylids are derived from marine ancestors. *Morone americana* in some areas is anadromous, while in others it is confined to fresh water. It would be of interest to follow the life cycle of *P. subsolana* experimentally, using hosts from strictly marine and freshwater populations to study saltwater tolerance in both adult and larval stages.

Beverley-Burton et al. (1986) considered *Aristocleidus hastatus* Mueller, 1936 found on *M. saxatilis* taken in the Peace River, Florida (possibly in brackish water) to be phylogenetically unrelated to other ancyrocephalids parasitizing freshwater fishes of the Nearctic. A study of the type material of *A. hastatus* showed the species was distinct and valid, but it is frustrating that it has not been found since the 1930s. The suggestion made by Beverley-Burton et al. (1986) that *Aristocleidus* may have phylogenetic affinities with ancyrocephalids parasitizing serranids has yet to be explored. However, Beverley-Burton and Klassen (1990), in their cladistic analysis of ancyrocephalids from Nearctic freshwater fishes, included *A. hastatus* in a clade (Group A) together with seven species all of uncertain taxonomic status. The phylogenetic relationships within this clade can be resolved only after the study of new material.

Centrarchidae (sunfishes)

Burr and Mayden (1992) noted there are 32 species in nine genera assigned to this family, which is generally considered to be enzootic and to have evolved in the Mississippi drainage. Burr and Mayden (1992) regarded the pygmy sunfish as a separate family (Elassomatidae) rather than a primitive group of centrarchids. The elassomatids may well be paraphyletic and their ancyrocephalid parasites, although originally assigned to *Urocleidus*, were considered incertae sedis by Wheeler and Beverley-Burton (1989*a*) and comprise the Group B of Beverley-Burton and Klassen (1990). Thus, there is a paucity of taxonomic information for both the hosts and their parasites and they are not considered further in the present paper.

Most (62) of the more than 100 known Nearctic ancyrocephalid species parasitize centrarchids. Gusev (1978) speculated that the ancyrocephalids parasitizing these Nearctic fishes had evolved from a marine ancestor common to those ancyrocephalids parasitizing serranids. Branson and Moore (1962) suggested the Centrarchidae arose from

a line of marine serranids that invaded fresh water during or before the Eocene. However, the supposed sister group relationship proposed by Branson and Moore (1962) of the Centrarchidae and the Serranidae was not upheld by Johnson (1984) and therefore, a marine origin for the centrarchids cannot be substantiated, and the family is regarded as enzootic by Burr and Mayden (1992).

Based on the hypothesis of Branson and Moore (1962), Beverley-Burton and Suriano (1981) postulated that an ancestral ancyrocephalid parasitizing serranids gave rise to the *Onchocleidus* group on centrarchids. Wheeler and Beverley-Burton (1989*b*), having established the monophyly of *Onchocleidus*, synoptically reviewed the feasibility of the origin of *Onchocleidus* on a marine perciform with subsequent colonization of freshwater centrarchids and concluded the concept must remain conjectural until further studies on a wide spectrum of ancyrocephalids from marine perciforms have been completed. However, Beverley-Burton and Klassen (1990) included *Onchocleidus* within the freshwater ancyrocephalids of North America, implying a freshwater origin. Thus, the origin of *Onchocleidus* remains problematic.

The other major group of ancyrocephalids found on centrarchids comprises 15 species in six genera, all of which have articulating haptoral bars, and which has been recognized as monophyletic (Beverley-Burton and Klassen 1990). A phylogeny for this articulating-bar group was presented by Klassen and Beverley-Burton (1988*a*) and the possibility of host–parasite coevolution was explored by Klassen and Beverley-Burton (1988*b*). Although Klassen and Beverley-Burton (1988*b*) worked with a host cladogram obtained from the dendrogram presented by Avise and Smith (1977), a satisfactory phylogeny for the centrarchid genera has not been generated. It was concluded by Klassen and Beverley-Burton (1988*b*) that cospeciation between the articulating-bar group of ancyrocephalids and their hosts was not evident but host-switching by ecological association was important.

The ancyrocephalids parasitizing centrarchids were recognized by Beverley-Burton and Klassen (1990) as a speciose, paraphyletic assemblage. These authors noted that there seems to be support for the hypothesis that the main radiation of the Nearctic ancyrocephalids occurred on the Centrarchidae, although their distribution among centrarchids appears to be dominated by host-switching.

Conclusions

Although the present paper includes comment on relatively few of the families (Table 1) listed by Burr and Mayden (1992), it is possible to draw several conclusions. Cone (1978) suggested that at least some of the Nearctic ancyrocephalids were of "mixed origins," i.e., were enzootic or had dispersed into the Nearctic with their hosts. This is clearly demonstrated in the examples chosen, in the present paper. Instances of vicariance, host-switching, and coevolution are also illustrated.

It is noteworthy that all the major Nearctic host taxa parasitized by ancyrocephalids were present at about the same geologic time and can be included in the older Nearctic fauna of at least Eocene age (Beverley-Burton and Klassen 1990).

The effect of vicariance is shown in the case of the sturgeons (Acipenseridae), where the fragmentation of Laurasia with the separation of the Nearctic and the Palearctic has led to the disjunct distribution of this group of fishes and their parasites (*Diclybothrium* spp.). A case of host-switching (*Diclybothrium hamulatum* from *Acipenser* sp. to *Polyodon spathula*) may have occurred in the Nearctic. Although the origin of *Pellucidhaptor* is uncertain, it is evident there has been considerable speciation on the Nearctic catostomids and, when suitable phylogenetic analyses of both hosts and parasites are available, it will be interesting to see if coevolution can be demonstrated. In contrast, *Pseudomurraytrema* is thought to be enzootic in the Nearctic and that one species, P. *asiaticus*, has dispersed into the eastern Palearctic on an ancestral catostomid host. Dispersal of hosts has also been suggested to explain the presence of *Urocleidus* on the large percids and *Aethycteron* on the darters, with both genera having been derived in Eurasia from an *Urocleidus*-like ancestor. The percopsids which are enzootic may have acquired *Urocleidus* from the large percids by host-switching.

The origin of the ancyrocephalid genus *Salsuginus*, which parasitizes top minnows, live bearers, and pupfishes, is unresolved. It has been suggested that an ancestral lineage evolved on marine atheriniform fishes, some of which moved into brackish–fresh water in ecological association with the present host taxa, and that host-switching occurred. An alternative hypothesis is that *Salsuginus* has a freshwater origin, as the genus is a monophyletic clade within the Nearctic freshwater ancyrocephalids.

The temperate basses, Moronidae, are enzootic, occurring in fresh water and brackish water of the eastern Nearctic. *Pauciconfibula* is probably derived from an ancestral microcotylid that parasitized a marine fish. In contrast, the four *Onchocleidus* spp. parasitizing these fishes are considered to have become associated with their hosts with four separate occurrences of host-switching from centrarchids.

Evidence to support coevolution is at present scanty. In the case of *Pellucidhaptor* spp. on their catostomid hosts, further research is needed to provide a workable parasite phylogeny. It is possible that an ancestral *Ligictaluridus* on ictalurids was transferred by host switching from centrarchids followed by coevolution, which can be substantiated for two-thirds of the described *Ligictaluridus* spp. The ancyrocephalids parasitizing the Nearctic freshwater fishes as a whole, and in particular the Centrarchidae, show no conclusive evidence for cospeciation with their hosts because of introgressive hybridization among the hosts that may have masked any coevolutionary pattern that may have been present. Beverley-Burton and Klassen (1990) supported the hypothesis that the main radiation of the Nearctic ancyrocephalids occurred on centrarchids but believed the distribution of these parasites on these fishes was dominated by host-switching.

Beverley-Burton and Klassen (1990) identified three major areas for ongoing research that remain pertinent at the present time: further taxonomic studies on the Nearctic ancyrocephalids comprising the unresolved assemblages identified in the present work; a comprehensive study of Holarctic ancyrocephalids, with particular reference to the possible sister-group relationship between the Nearctic and

Eurasian ancyrocephalids on percids; and a study of marine ancyrocephalids, particularly those parasitizing serranids, to help interpret the early (Cretaceous) phylogenetic relationships among these animals and those of the Nearctic and Palearctic faunas.

Acknowledgements

Part of this article was the major component of a paper co-authored with Dr. D.C. Kritsky, Idaho State University, on "The Origins of the Freshwater Monogenea of the Neotropical and Nearctic Regions," which was presented at the Second International Symposium on Monogenea in Montpellier in 1993. I gratefully acknowledge Dr. Kritsky's invaluable and sustained effort in the preparation of the present manuscript. I am also indebted to Dr. T.A. Wheeler, University of Guelph, for his constructive criticism and Dr. I.D. Whittington, University of Queensland, for editorial comment. I thank Ms. L.A. Chisholm, University of Guelph, who has given continual technical support throughout the preparation of this paper. This work was supported by Natural Sciences and Engineering Council of Canada operating grant No. 801-81.

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Organisation et développement du système osmorégulateur chez les Monogenea

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Résumé : À partir des données de la littérature, les auteurs tentent une synthèse de l'état des connaissances sur le système osmorégulateur de l'oncomiracidium des Monogenea et sur son évolution au cours du développement postlarvaire. Chez l'oncomiracidium, ils définissent deux types caractérisant respectivement les Monopisthocotylea et les Polyopisthocotylea. Dans chaque type, ils reconnaissent plusieurs états d'après le nombre et la disposition des protonéphridies. Chez les Monopisthocotylea ces états paraissent en accord avec la complication ontogénique du système. Le développement du sytème osmorégulateur du *Diplectanum aequans* (Wagener, 1857) est considéré comme représentatif des Monopisthocotylea. Les auteurs signalent, chez les Polyopisthocotylea, une différence entre le développement postlarvaire du système osmorégulateur des Microcotylidae et des Gastrocotylidae. Les résultats de cette synthèse semblent apporter de nouveaux arguments en faveur de l'hypothèse d'une origine polyphylétique des Monopisthocotylea et monophylétique des Polyopisthocotylea.

Abstract: On the basis of data obtained from the literature, an attempt is made to synthesize current knowledge on the oncomiracidial osmoregulatory system of the Monogenea and its evolution during post-larval development. At the oncomiracidial level, two types characteristic respectively of the Monopisthocotylea and Polyopisthocotylea are defined. In each type, many states are recognized, based on the number and disposition of protonephridia. In the Monopisthocotylea these states appear to be in harmony with the ontogenic complication of the system. The development of the osmoregulatory system of *Diplectanum aequans* (Wagener, 1857) is considered representative of the Monopisthocotylea. In the Polyopisthocotylea, a difference is pointed out between the post-larval development of the osmoregulatory system of the Microcotylidae and the Gastrocotylidae. The results of this synthesis add new support to the hypothesis of the polyphyletic origin of the Monopisthocotylea and the monophyletic origin of the Polyopisthocotylea.

Introduction

Le système osmorégulateur (ou « excréteur » selon les auteurs) des Monogenea comprend deux ensembles de protonéphridies disposées symétriquement par rapport au plan médio-sagittal. Celles-ci sont reliées par une arborisation de canalicules et de canaux avec le tronc principal ouvert à l'extérieur. L'ultrastructure de la protonéphridie (cellule flamme ou flamme vibratile) a été parfaitement étudiée par Rohde (1973) et Rohde et al. (1989). Du type Plathelminthe, elle ne semble pas présenter de variations très importantes à l'intérieur de la classe des Monogenea. Par contre, chez l'oncomiracidium, les données de la littérature mettent en évidence, selon les espèces, des différences sensibles dans le nombre de protonéphridies et dans la disposition des canalicules et des canaux.

Le développement postlarvaire du système osmorégulateur est encore peu étudié. Dans le présent travail, nous tentons de dresser une synthèse des connaissances actuellement disponibles.

Zeller (1872, cité par Bykhovsky, 1957) semble être le premier à signaler des canaux excréteurs chez l'adulte du *Polystoma integerrimum* (Froelich, 1791). Halkin (1902) décrit des canaux de même nature chez l'oncomiracidium du *P. integerrimum*. Jahn et Khun (1932) publient la première description du système osmorégulateur complet d'un oncomiracidium, en l'occurrence le *Neobenedenia melleni* (MacCallum, 1927) (Capsalidae). Bykhovsky (1957) rassemble, avec des données originales, les observations ponctuelles réalisées jusqu'alors sur le système osmorégulateur des Monogenea. Llewellyn (1963) décrit le système osmorégulateur chez quelques oncomiracidia et présente une vision schématique de la disposition de ce système.

À partir de cette date, l'anatomie du système osmorégulateur a été décrite chez de nombreux monogènes. On la connaît, en totalité ou en partie, chez l'oncomiracidium de 32 Monopisthocotylea et de 41 Polyopisthocotylea.

Reçu le 8 juin 1994. Accepté le 15 décembre 1994.
J12664

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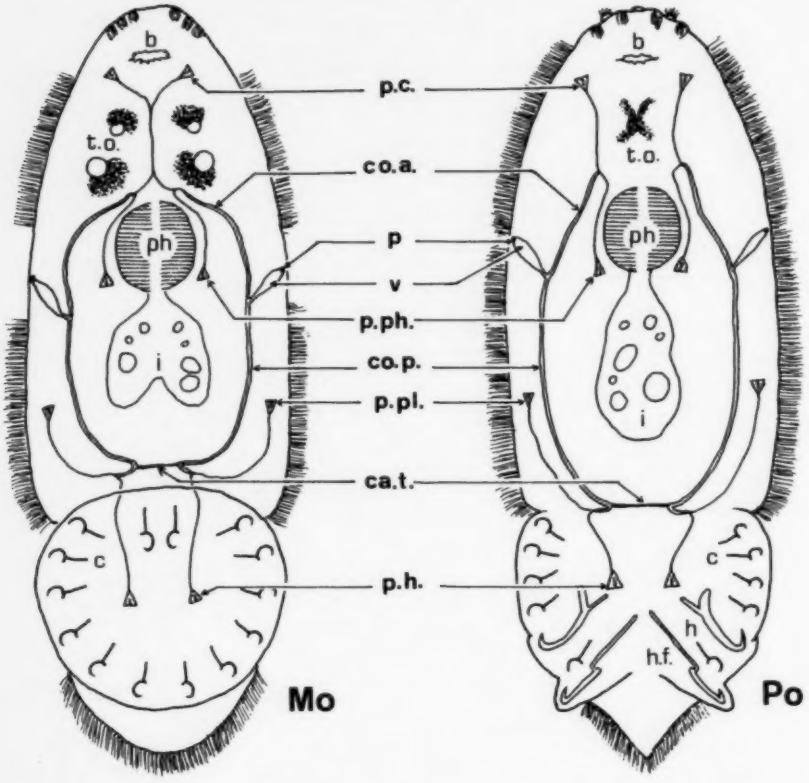
Fig. 1. Organisation générale de l'oncomiracidium des deux sous-classes des Monogenea : Monopisthocotylea (Mo) et Polyopisthocotylea (Po). *b*, bouche; *c*, crochetons latéraux; ca. *t*., canal transverse; co. *a*., collecteur antérieur; co. *p*., collecteur postérieur; *h*, hamuli; h. f., hamuli en fléau; *i*, intestin; *p*, pore excréteur; p. c., protonéphridie céphalique; p. h., protonéphridie haptoriale; p. ph., protonéphridie pharyngienne; p. pl., protonéphridie pleurale; *ph*, pharynx; t. o., tache oculaire; *v*, vessie.



Organisation générale du système osmorégulateur larvaire

À partir des données disponibles, on peut établir de la manière suivante le plan fondamental du système osmorégulateur de l'oncomiracidium. Il comprend huit protonéphridies disposées symétriquement, quatre de part et d'autre du plan médio-sagittal. On distingue ainsi quatre paires de protonéphridies que, d'après leur position dans le corps, Euzet et Combes (1969) nomment, de l'avant vers l'arrière, céphaliques, pharyngiennes, pleurales et haptoriales (fig. 1). De chaque côté du corps, le canalicule issu de la protonéphridie céphalique rejoint le canalicule de la protonéphridie pharyngienne. Leur union donne naissance au collecteur antérieur descendant. De la même manière, le canalicule issu de la protonéphridie pleurale s'unit, au niveau de la limite corps–hapteur, au canalicule de la protonéphridie haptoriale. Il se forme ainsi le collecteur postérieur ascendant. Dès leur formation, un petit canal transverse met en communication les collecteurs postérieurs droit et gauche. Les deux collecteurs (antérieur et postérieur) se rejoignent et donnent le tronc commun, parfois dilaté en vessie, s'ouvrant à l'extérieur par un pore « excréteur ». Cet orifice est situé latéro-dorsalement légèrement en arrière ou au niveau de la région pharyngienne (fig. 1).

En nous inspirant de la technique utilisée pour décrire le système osmorégulateur (excréteur) de la cercaire des Digenea, nous proposons de définir le plan fondamental du système osmorégulateur chez l'oncomiracidium des Monogenea par la formule 2 [(1 + 1) + (1 + 1)]. Nous nommons sous-ensemble antérieur les protonéphridies céphaliques et pharyngiennes, leurs canalicules et le collecteur antérieur et, sous-ensemble postérieur, les protonéphridies pleurales et haptoriales, leurs canalicules et le collecteur postérieur.

Chez certains Monopisthocotylea, on a décrit, sur le trajet des collecteurs antérieurs et postérieurs, des pinceaux de cils vibratiles, ressemblant à une protonéphridie et appelés flamulae collectoris. Leur rôle est mal connu. Le battement de ces groupes de cils pourrait servir à accélérer la circulation du contenu des collecteurs.

Dans l'oncomiracidium de la majorité des Polyopisthocotylea, les canalicules céphaliques droit et gauche sont séparés (fig. 1). Malmberg (1986) considère cette

disposition comme typique pour la classe des Monogenea. En fait, dans l'oncomiracidium des Monopisthocotylea, ces canalicules droit et gauche sont unis (fig. 1) ou, dans certains cas, étroitement accolés dans le plan médio-sagittal sur une longue partie de leur trajet. On peut ainsi différencier, pour le plan d'organisation du système osmorégulateur de l'oncomiracidium, deux types que nous désignons par Po et Mo d'après les premières lettres du nom des deux ensembles taxonomiques distingués ci-dessus.

Le système osmorégulateur ayant une symétrie bilatérale, les descriptions qui vont suivre se rapportent à un hémicorps. La couverture ciliée n'est pas représentée dans l'illustration schématique des oncomiracidia.

Monopisthocotylea

Nous distinguons, dans cette sous-classe, pour le type Mo, cinq états d'après le nombre de protonéphridies qui composent le système.

Le premier état (Mo1) (fig. 2A), correspondant au type Mo tel que nous l'avons schématisé, a été décrit chez :

Ancyrocephalus parvus Linton, 1940 (Ancyrocephalidae) par Kingston et al. (1969),

Rhamnocercus bairdiella Hargis, 1955 (Diplectanidae, Rhamnocercinae) par Kingston et al. (1969),

Heterocotyloides pricei (Pearse, 1949) (Monocotylidae) par Kingston et al. (1969),

Lamellodiscus sp. (Diplectanidae, Lamellodiscinae) par Suriano (1971),

Ergenstrema mugilis Paperna, 1964 (Ancyrocephalidae) par Lambert (1977),

Furnestinia echeneis (Wagener, 1857) (Diplectanidae, Lamellodiscinae) par Oliver (1987).

Chez le *Merizocotyle* sp. (Monocotylidae), Kearn (1968) décrit un système sans protonéphridie pleurale. La comparaison avec le système osmorégulateur d'un autre Monocotylidae, l'*Heterocotyloides pricei*, amène à penser que cette protonéphridie n'a peut-être pas été observée.

Un deuxième état (Mo2) (fig. 2B) est caractérisé par la formation, sur le collecteur antérieur, d'une anse dirigée vers l'avant et par l'apparition d'une deuxième protonéphridie pharyngienne. Oliver (1987) nomme ces protonéphridies pré- et post-pharyngiennes. Cette disposition, représentée par la formule 2 [(1 + 2) + (1 + 1)], a été signalée chez :

Dactylogyrus sp. (Dactylogyridae) par Malmberg (1956),

Dicrumenia bychowskyi Mamaev, 1969 (Calceostomatidae) par Euzet et Ktari (1970*b*),

Calceostoma calceostoma (Wagener, 1857) (Calceostomatidae) par Euzet et Ktari (1970*b*),

Calceostomella inermis (Parona et Perugia, 1889) (Calceostomatidae) par Euzet et Ktari (1970*b*),

Diplectanum aequans (Wagener, 1857) (Diplectanidae, Diplectaninae) par Oliver (1987).

Deux oncomiracidia, appartenant peut-être à cet état, doivent être revus : (*i*) celui du *Ligophorus vanbenedenii* (Parona et Perugia, 1890) (Ancyrocephalidae), qui est décrit par Suriano (1971) avec une anse sur le trajet du collecteur antérieur mais avec une seule protonéphridie pharyngienne occupant la position de la protonéphridie postpharyngienne, et (*ii*) celui de l'*Acanthocotyle lobianchi* Monticelli, 1888 (Acanthocotylidae), qui possède deux protonéphridies pharyngiennes et l'anse du collecteur antérieur, mais chez lequel Kearn (1967) a noté une seule protonéphridie dans la partie postérieure du corps (voir plus loin la discussion à propos de l'attribution de cette protonéphridie au groupe haptorial ou au groupe pleural).

Kearn (1993) décrit l'oncomiracidium de l'*Enoplocotyle kidakoi* Kearn, 1993 avec seulement quatre protonéphridies. La situation des deux protonéphridies antérieures correspond à celle des protonéphridies pré- et post-pharyngienne. Est-ce que les protonéphridies céphaliques n'ont pas été observées? Si elles existent, le système osmorégulateur de cet oncomiracidium correspond à l'état Mo2.

Cone (1979) signale chez l'oncomiracidium de l'*Urocleidus adspectus* Mueller, 1936 (Ancyrocephalidae), un système osmorégulateur dont on pourrait schématiser la disposition par la formule 2[(1 + 1) + (1 + 2)]. Mais le trajet des canaux de cette larve doit être vérifié car il ne peut être déduit de ce que l'on connaît dans le type fondamental Mo.

Un troisième état (Mo3) (fig. 2C) se distingue par la formation d'une deuxième protonéphridie haptoriale. Cet état, correspondant à la formule 2[(1 + 2) + (1 + 2)], a été décrit par Kearn (1970) chez deux Monocotylidae, le *Dictyocotyle coeliaca* Nybelin, 1941 et le *Calicotyle kroyeri* Diesing, 1850.

La disposition du système osmorégulateur de l'oncomiracidium du *Capsala onchidiocotyle* (Setti, 1899) (Capsalidae) (présent travail) permet de définir un quatrième état (Mo4) (fig. 2D). Nous le caractérisons par la formule 2[(2 + 2) + (1 + 2)].

Un cinquième état (Mo5) (fig. 2E et 2F) peut être défini par l'apparition, à partir de l'état précédent, de deux nouvelles protonéphridies haptoriales. Cette disposition, correspondant à la formule 2[(2 + 2) + (1 + 4)], a été décrite chez les Capsalidae suivants :

Entobdella soleae (Van Beneden et Hesse, 1863) (Capsalidae, Benedeniinae) par Kearn (1963*a*),

Trochopus pini (Van Beneden et Hesse, 1863) (Capsalidae, Trochopodinae) par Suriano (1971),

Entobdella hippoglossi (Müller, 1776) (Capsalidae, Benedeniinae) par Kearn (1974),

Entobdella diadema (Monticelli, 1902) (Capsalidae, Benedeniinae) par Llewellyn et Euzet dans Llewellyn (1963).

Bien que Suriano (1971) décrive l'oncomiracidium du *Benedenia monticellii* (Parona et Perugia, 1895) (Benedeniinae) avec trois protonéphridies pleurales, nous le classons avec les cinq espèces précédentes (Mo4 et Mo5). La formule de ce système osmorégulateur peut s'écrire 2[(2 + 2) + (3 + 4)].

Nous incluons également, dans l'ensemble caractérisé par un système osmo-régulateur à l'état Mo5, l'oncomiracidium du *Neobenedenia melleni* (MacCallum, 1927) (Benedeniinae), chez lequel Jahn et Kuhn (1932) décrivent un système différent de Mo5 par la présence d'une protonéphridie haptoriale supplémentaire. On a alors la formule 2[(2 + 2) + (1 + 5)].

Notons que chez l'oncomiracidium de trois de ces Capsalidae (*T. pini*, *E. hippoglossi* et *B. monticellii*), on a figuré les canalicules céphaliques non pas unis, mais seulement contigus sur le plan médio-sagittal. Nos observations sur l'oncomiracidium du *B. monticellii* confirment, à ce sujet, les données de Suriano (1971).

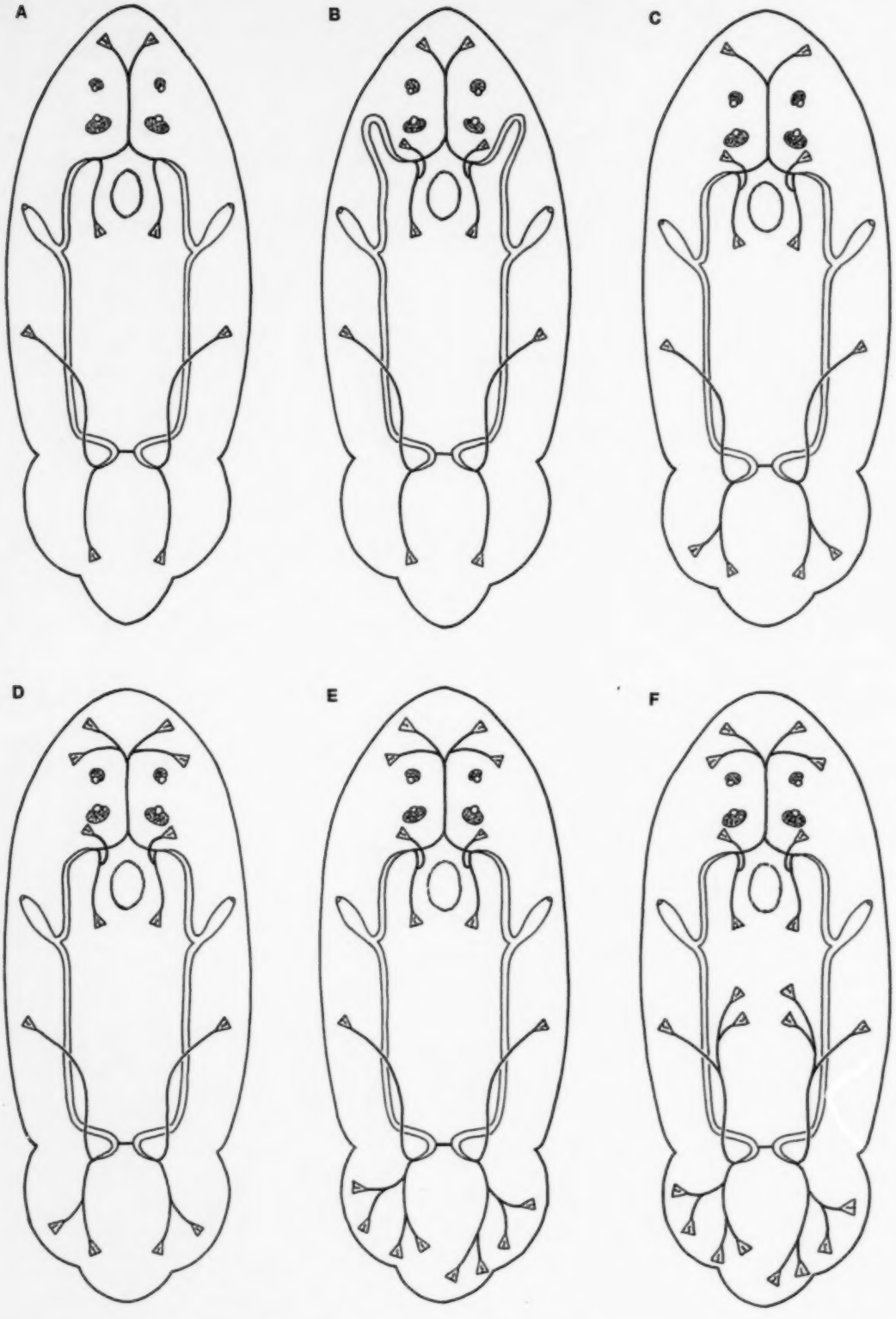
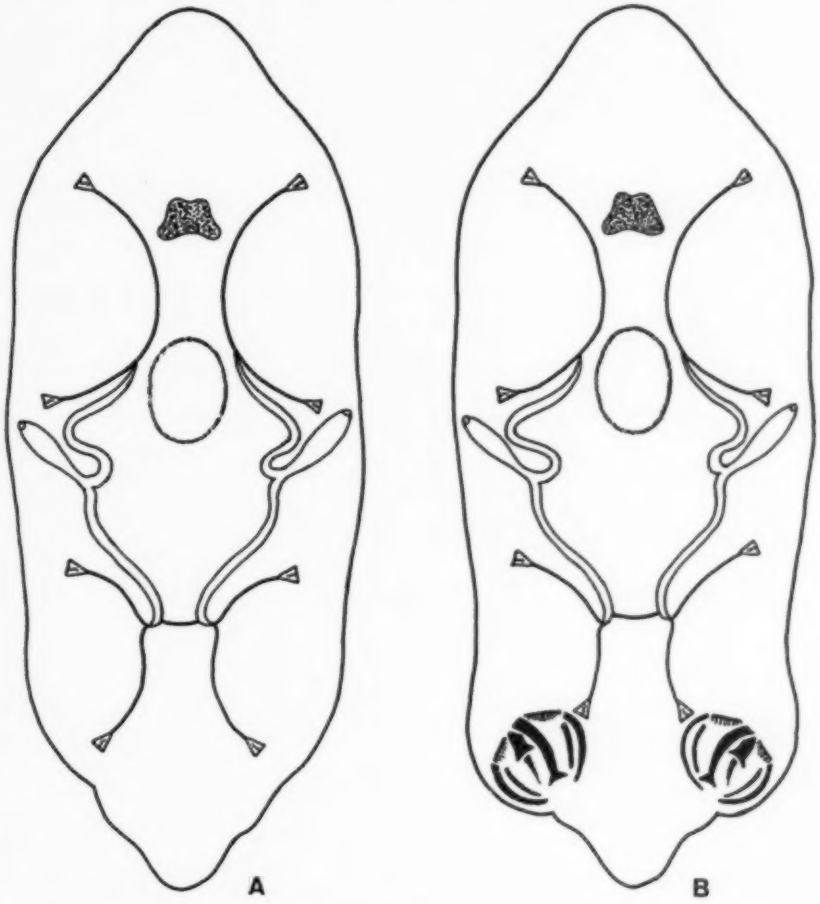

A
B
C
D
E
F

Fig. 2. Schéma du système osmorégulateur d'un oncomiracidium de Monopisthocotylea. États Mo1, *Furnestinia echeneis* (d'après Oliver 1987, modifié) (A), Mo2, *Diplectanum aequans* (d'après Oliver 1987, modifié) (B), Mo3, *Calicotyle kroyeri* (d'après Kearn 1970, modifié) (C), et Mo4, *Capsala onchidiocotyle* (cette étude) (D); Capsalidae à l'état Mo5 : *Entobdella soleae* (d'après Kearn 1963*a*, modifié) (E) et *Benedenia monticelli* (d'après Suriano 1971, modifié) (F).

Fig. 3. Schéma du système osmorégulateur d'oncomiracidia de Polyopisthocotylea à l'état Po1. Oncomiracidium sans pince (A) et avec la première paire de pinces (B).



Le système osmorégulateur de plusieurs oncomiracidia de Capsalidae a été décrit sans protonéphridie pleurale. Les données recueillies dans la littérature permettent d'établir les formules suivantes :

2[(1 + 2) + (0 + 2)] chez le *Capsala martinieri* Bosc, 1811 (Capsalinae) d'après Kearn (1963*b*),

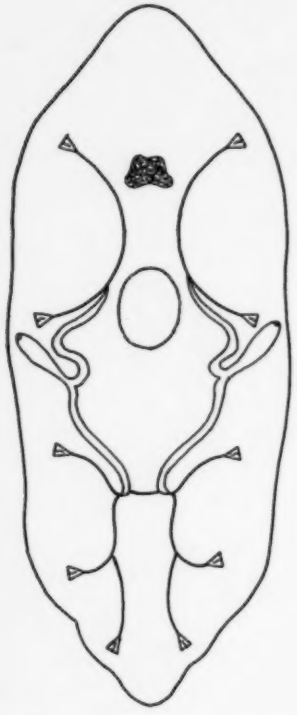
2[(1 + 2) + (0 + 4)] chez le *Benedenia seriolae* (Yamaguti, 1934) (Benedeniinae) d'après Kearn et al. (1992*b*), chez le *B. lutjani* Whittington et Kearn, 1993 (Benedeniinae) d'après Whittington et Kearn (1993) et le *B. rohdei* Whittington, Kearn & Beverley-Burton, 1994 (Benedeniinae) d'après Whittington et al. (1994),

2[(2 + 2) + (0 + 4)] chez l'*Encotyllabe caballeroi* Velasquez, 1977 et chez l'*Encotyllabe caranxi* Lebedev, 1967 (Encotyllabinae) d'après Whittington et Kearn (1992).

Si cette absence de protonéphridie pleurale était confirmée, cette disposition correspondrait à un nouvel état qui se situerait entre Mo3 et Mo4.

Malmberg (1956, 1970) décrit des flamulae collectoris dans le système osmorégulateur de plusieurs espèces du genre *Gyrodactylus*. La présence de telles flamulae a été observée dans ce système chez quelques larves de Monopisthocotylea. En 1956, Malmberg signale la présence de deux flamulae dans le collecteur antérieur et une flamula dans le collecteur postérieur chez un « jeune stade » du *Dactylogyrus* sp., parasite du *Leuciscus erythrophthalmus*. Reprenant cette étude, Malmberg et Malmberg (1970) indiquent qu'il s'agit là de la disposition d'un stade larvaire (oncomiracidium?). Kingston et al. (1969) signalent un collecteur antérieur cilié chez l'oncomiracidium de l'*Ancyrocephalus parvus* Linton, 1940 et de l'*Heterocotyloides pricei* (Pearse,1949). En 1977, Lambert observe

Fig. 4. Schéma du système osmorégulateur d'un oncomiracidium de Polyopisthocotylea à l'état Po2 : *Serranicotyle labracis* (d'après Oliver 1989, modifié).



trois flamulae collectoris dans le collecteur antérieur de l'oncomiracidium de l'*Ergenstrema mugilis* Paperna, 1964, et cinq dans le collecteur postérieur. Oliver (1987) signale une flamula dans le collecteur antérieur et une dans le collecteur postérieur de l'oncomiracidium du *Furnestinia echeneis* (Wagener, 1857) et seulement deux flamulae dans le collecteur antérieur de l'oncomiracidium du *Diplectanum aequans* (Wagener, 1857).

Ces variations, ainsi que l'absence de ces formations chez les autres oncomiracidia, devraient être confirmées par de nouvelles recherches.

Polyopisthocotylea

Le nombre de protonéphridies nous permet de distinguer quatre états.

Un premier état (Po1) (fig. 3A), correspond au plan fondamental Po avec les canalicules céphaliques séparés, tel que nous l'avons défini plus haut. Représenté par la formule 2[(1 + 1) + (1 + 1)], cet état a été signalé, par ordre chronologique, chez :

Gastrocotyle trachuri Van Beneden et Hesse, 1863 (Gastrocotylidae) par Llewellyn (1963),

Polylabris tubicirrus (Paperna et Khon, 1964) (= *P. diplodi* Euzet et Cauwet, 1967) (Microcotylidae, Prostatomicrocotyline) par Euzet et Cauwet (1967),

Metamicrocotyla cephalus (Azim, 1939) (Microcotylidae, Metamicrocotylinae) par Euzet et Combes (1969),

Atrispinum salpae Parona et Perugia, 1890 (Microcotylidae, Atriasterinae) par Ktari (1969),

Pedocotyle minima Hargis, 1955 (Diclidophoridae) par Kingston et al. (1969),

Nudaciraxine gracilis (Linton, 1940) (= *Axine gracilis* Linton, 1940) (Axinidae) par Kingston et al. (1969),

Intracotyle hannibali (Euzet et Ktari, 1970) (= *Heteraxinoides hannibali* Euzet et Ktari, 1970) (Microcotylidae, Metamicrocotylinae) par Euzet et Ktari (1970a),

Microcotyle panceri Sonsino, 1891 (Microcotylidae, Microcotylinae) par Ktari (1970),

Rhinecotyle crepitacula Euzet et Trilles, 1960 (Gastrocotylidae) par Euzet et Wahl (1970),

Discocotyle sagittata (Leuckart, 1842) (Discocotylidae) par Owen (1970),

Pseudoaspinatrium gallieni (Euzet et Ktari, 1971) (= *Aspinatrium gallieni* Euzet et Ktari, 1971) (Microcotylidae, Microcotylinae) par Euzet et Ktari (1971),

Pseudoaspinatrium euzeti (Ktari, 1971) (= *Aspinatrium euzeti* Ktari, 1971) (Microcotylidae, Microcotylinae) par Ktari (1971*a*),

Pagellicotyle mormyri (Lorenz, 1878) (= *Microcotyle mormyri* Lorenz, 1878) (Microcotylidae, Atriasterinae) par Ktari (1971*b*),

Bivagina alcedinis (Parona et Perugia, 1890) (= *Microcotyle alcedinis* Parona et Perugia, 1890) (Microcotylidae, Microcotylinae) par Ktari (1971*b*),

Pauciconfibula trachini (Parona et Perugia, 1890) (= *Microcotyle trachini* Parona et Perugia, 1889) (Microcotylidae, Microcotylinae) par Ktari (1971*b*),

Mazocraes sardinellae Euzet et Ktari dans Ktari, 1971 (Mazocraeidae) par Ktari (1971b),

Chauhanea mediterranea Euzet et Trilles, 1960 (Gastrocotylidae) par Ktari (1971*b*),

Heteraxine seriolae (Ishii, 1936) (Axinidae, Heteraxininae) par Ktari (1971*b*),

Neohexostoma euthynni (Meserve, 1938) (Hexostomatidae) par Ktari (1971*b*),

Pyragraphorus hollisae Euzet et Ktari, 1972 (Pyragraphoridae) par Euzet et Ktari (1972),

Orbocotyle marplatensis Euzet et Suriano, 1975 (Diclidophoridae) par Euzet et Suriano (1975),

Sparicotyle chrysophrii (Van Beneden et Hesse, 1863) (= *Microcotyle chrysophrii* Van Beneden et Hesse, 1863) (Microcotylidae, Atriasterinae) par Euzet et Noisy (1979),

Microcotyle sebastis Goto, 1894 (Microcotylidae, Microcotylinae) par Thoney (1986),

Heteraxinoides xanthophilis (Hargis, 1956) (Axinidae, Heteraxininae) par Thoney (1988),

Kuhnia scombri (Kuhn, 1829) (Mazocraeidae, Mazocraeinae) par Whittington et Kearn (1990),

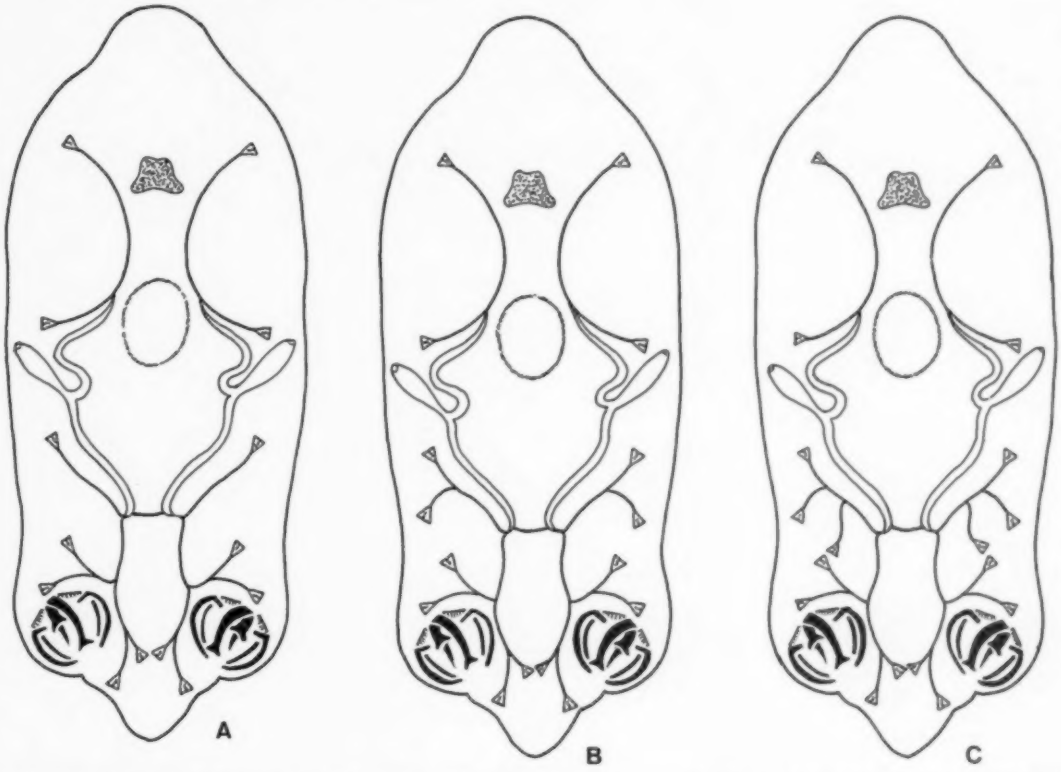
Kuhnia sprostonae Price, 1961 (Mazocraeidae, Mazocraeinae) par Whittington et Kearn (1990),

Grubea cochlear Diesing, 1858 (Mazocraeidae, Grubeinae) par Whittington et Kearn (1990),

Heteraxine heterocerca (Goto, 1894) (Axinidae, Heteraxininae) par Kearn et al. (1992*a*).

Euzet et Raibaut (1961) n'ont pas décrit de protonéphridie pleurale chez l'oncomiracidium du *Vallisia striata* Parona et Perugia, 1890 (Gastrocotylidae). Ce travail étant l'un des premiers consacré à l'étude du système osmorégulateur,

Fig. 5. Schéma du système osmorégulateur chez trois formes de l'oncomiracidium du *Microcotyle mugilis*, Polyopisthocotylea à l'état Po3. Oncomiracidium avec une (A), deux (B) et trois (C) paires de protonéphridies pleurales (d'après Euzet et Combes 1969, modifié).



il est probable que la protonéphridie pleurale n'a pas été repérée. Cependant, d'après Whittington et Kearn (1989), la même absence de protonéphridie pleurale existe chez l'oncomiracidium du *Plectanocotyle gurnardi* (Van Beneden et Hesse, 1863).

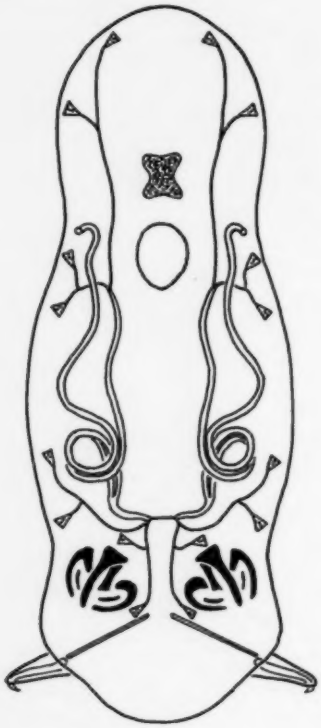
Kingston et al. (1969) décrivent, dans l'oncomiracidium du *Macrovalitrematoides micropogoni* (Pearce, 1949), du *Cynoscionicola heteracantha* (Manter, 1938) et du *Heteraxinoides xanthophilis* (Hargis, 1956), un système osmorégulateur avec seulement trois protonéphridies par hémicorps mais, d'après l'illustration, on ne peut préciser quelle est celle qui semble manquer. Enfin, chez le *Mazocraeoides hargisi* Price, 1961 ils décrivent cinq paires de protonéphridies, mais tous les canalicules n'étant pas notés, la disposition peut répondre soit à la formule 2[(2 + 1) + (1 + 1)] soit à 2[(1 + 1) + (2 + 1)].

Whittington (1987) décrit, chez l'oncomiracidium du *Rajonchocotyle emarginata* (Olsson, 1876) et celui de l'*Hexabothrium appendiculatum* (Kuhn, 1829) (Hexabothriidae, Hexabothriinae), un système osmorégulateur avec les canaux céphaliques unis et sans anastomose entre les collecteurs postérieurs. Toutefois, chez l'*Erpocotyle catenulata* (Guberlet, 1933), (Hexabothriidae, Hexabothriinae) Maillard (1966) figure les canaux céphaliques séparés et précise : « On trouve sur l'axe médian du corps deux canaux parallèles recevant 2 protonéphridies avant de se séparer en avant du pharynx ». Les larves de cette famille doivent donc être revues pour savoir si la disposition de leur système osmorégulateur correspond au type Monopisthocotylea (Mo) avec canalicules céphaliques unis, ou, comme on peut le subodorer d'après la position systématique de la famille des Hexabothriidae, au type Polyopisthocotylea (Po) avec canaux céphaliques séparés.

Chez l'oncomiracidium de trois espèces, répondant à un système osmorégulateur à l'état Po1, on a décrit la présence, dans le hapteur, de la première paire de pinces (fig. 3B). Il s'agit de l'oncomiracidium du *Polylabris tubicirrus* d'après Euzet et Cauwet (1967), du *Discocotyle sagittata* d'après Owen (1970), et du *Pagellicotyle mormyri* chez qui Ktari (1971*b*) mentionne l'existence d'oncomiracidia, les uns avec pinces, les autres sans.

Un deuxième état (Po2) (fig. 4) peut être caractérisé par l'apparition d'une deuxième protonéphridie haptoriale. Llewellyn (1963) a ainsi signalé, chez l'oncomiracidium du *Serranicotyle labracis* (Van Beneden et Hesse, 1863) (= *Microcotyle labracis* Van Beneden et Hesse, 1863), la présence de cinq protonéphridies sans toutefois donner ni leur position exacte ni le trajet des canaux et des collecteurs. Chez ce Microcotylidae, Oliver (1989) retrouve les cinq protonéphridies et précise que l'augmentation résulte de la présence d'une deuxième protonéphridie haptoriale. Cet état Po2 correspond ainsi à la formule 2[(1 + 1) + (1 + 2)].

Fig. 6. Schéma du système osmorégulateur d'un oncomiracidium de Polyopisthocotylea à l'état Po4 : Diplozoon gracile (d'après Euzet et Lambert 1971, modifié).

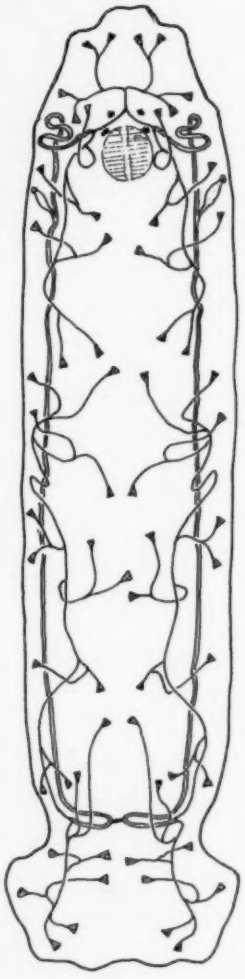


Euzet et Combes (1969) décrivent un troisième état (Po3) (fig. 5) chez l'oncomiracidium du *Microcotyle mugilis* Vogt, 1878 (Microcotylidae). Celui-ci est caractérisé par la présence de quatre protonéphridies haptoriales et d'un nombre variable (une, deux ou trois selon les individus) de protonéphridies pleurales. Les variations individuelles de cet état peuvent être représentées par les formules 2[(1 + 1) + (1 + 4)], 2[(1 + 1) + (2 + 4)] ou 2 [(1 + 1) + (3 + 4)].

Le quatrième état (Po4) (fig. 6) a été observé chez quatre espèces du genre *Diplozoon* Von Nordmann, 1832. Il est caractérisé par un dédoublement de toutes les protonéphridies. Ainsi, Euzet et Lambert (1971) chez le *Diplozoon gracile* Reichenbach-Klinke, 1961[1] puis Lambert et Denis (1982), chez le *D. nipponicum* Goto, 1891 et le *D. homoion* Bykhovsky et Nagibina, 1959, décrivent huit protonéphridies : deux céphaliques, deux pharyngiennes, deux pleurales et deux haptoriales. On peut représenter cette disposition par la formule 2 [(2 + 2) + (2 + 2)].

1. Le parasite du *Gobio gobio* et du *Phoxinus phoxinus*, identifié par Euzet et Lambert (1971) comme étant le *Diplozoon paradoxum*, a été par la suite rapporté au *Diplozoon gracile* en se basant sur le critère de la spécificité parasitaire (Le Brun et al. 1985).

Fig. 7. Schéma de la disposition du système osmorégulateur d'un jeune individu du *Ligophorus vanbenedenii* à 31 paires de protonéphridies (d'après Suriano 1971, modifié).

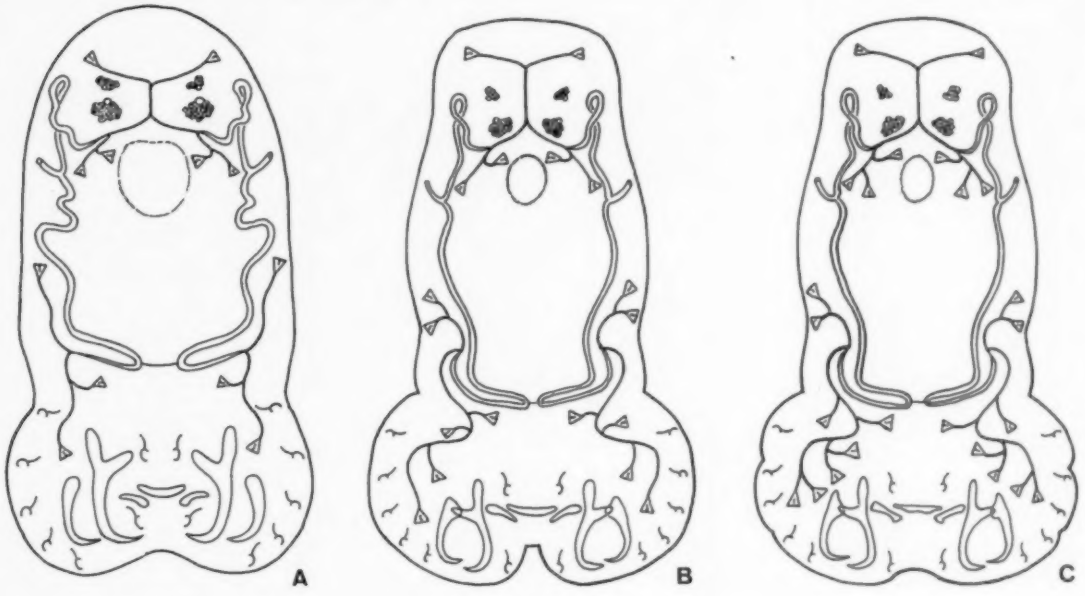


Chez le *Diplozoon paradoxum* Von Nordmann, 1832, parasite de l'*Abramis brama*, Bovet (1959, 1967) signale sept protonéphridies dont une seule haptoriale. Il est possible que la deuxième protonéphridie haptoriale n'ait pas été observée. La disposition des collecteurs, identique chez les quatre larves, est en effet caractérisée par un allongement très important du collecteur antérieur, lequel vient rejoindre le petit collecteur postérieur à la limite corps–hapteur. C'est à ce niveau qu'on peut observer le canal transverse réunissant les deux collecteurs postérieurs.

Développement postlarvaire du système osmorégulateur

Comme nous l'avons souligné plus haut, les données sur le développement postlarvaire du système osmorégulateur sont

Fig. 8. Schéma du développement postlarvaire du système osmorégulateur du *Diplectanum aequans*. État à 6 (A), 9 (B) et 12 (C) paires de protonéphridies (d'après Oliver 1987, modifié).



rares. Bykhovsky (1957) mentionne que la croissance générale du corps s'accompagne du développement parallèle des systèmes osmorégulateur et digestif qui deviennent de plus en plus complexes.

D'après le travail de Suriano (1971) sur le genre *Ligophorus*, nous savons que, chez les adultes, le nombre de protonéphridies est élevé. Variable selon les espèces, ce nombre paraît en rapport avec la taille et l'âge des individus. Suriano a ainsi compté, chez un jeune *Ligophorus vanbenedenii* (Parona et Perugia, 1890) 62 (2 × 31) protonéphridies réparties suivant la formule 2[(4 + 7) + (12 + 8)] (fig. 7).

Bien que la disposition ne soit pas toujours absolument symétrique, elle dérive du plan fondamental avec, dans chaque hémicorps, un sous-ensemble antérieur (groupe céphalique et groupe pharyngien) et un sous-ensemble postérieur (groupe pleural et groupe haptorial) inégaux par le nombre de protonéphridies.

Mais comment, à partir du plan fondamental larvaire à quatre protonéphridies par hémicorps, parvient-on au système compliqué de l'adulte?

On a décrit, aussi bien chez les Monopisthocotylea que chez les Polyopisthocotylea, une augmentation progressive du nombre de protonéphridies en particulier dans les régions pleurales et haptoriales. Notons que l'origine et le mode de formation d'une nouvelle protonéphridie restent inconnus.

Monopisthocotylea

Le travail d'Oliver (1987) sur l'état du système osmorégulateur chez quelques post-larves du *Diplectanum aequans* permet de proposer un schéma du développement de ce système. Nous discuterons plus loin l'évolution du système osmorégulateur chez les postlarves d'*Acanthocotyle lobianchi* Monticelli, 1890, telle que l'a présentée Kearn en 1967, car elle entre difficilement dans ce schéma.

Chez le *Diplectanum aequans* le système de l'oncomiracidium est à l'état Mo2, représenté par la formule 2[(1 + 2) + (1+ 1)].

Durant les premières phases du développement (formation, dans le hapteur, des crochets et de l'ébauche des barres transversales), on n'observe pas de transformation du système osmorégulateur. C'est seulement lorsque les trois barres transversales ont presque atteint leur morphologie définitive qu'apparaît une deuxième protonéphridie haptoriale (fig. 8A). La formule devient ainsi 2[(1 + 2) + (1 + 2)].

Immédiatement après, il se forme dans le hapteur, deux nouvelles protonéphridies haptoriales et, dans le corps, une deuxième protonéphridie pleurale (fig. 8B). Cette disposition répond à la formule 2[(1 + 2) + (2 + 4)].

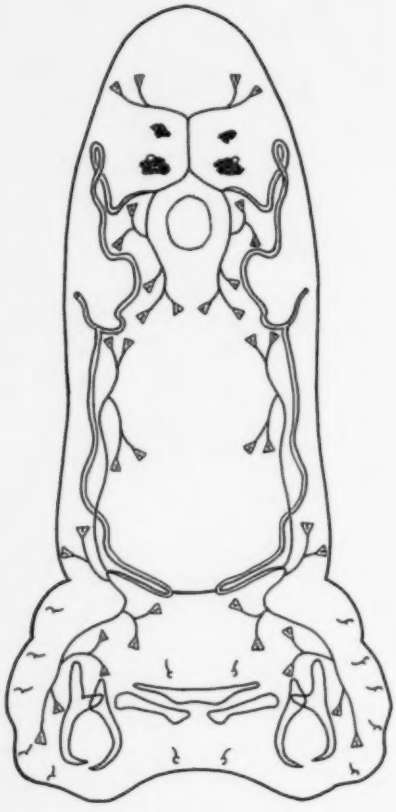
Le stade suivant est caractérisé par l'apparition d'une deuxième protonéphridie postpharyngienne et de deux nouvelles protonéphridies haptoriales fig. 8C). Cela peut être schématisé de la manière suivante : 2[(1 + 3) + (2 + 6)].

La complication ultérieure intéresse plutôt le sous-ensemble antérieur. Ainsi, chez un individu de 310 µm de longueur, on compte en tout 36 protonéphridies (fig. 9). Ce stade pourrait être représenté par la formule 2[(2 + 5) + (4 + 8)]. Notons qu'il existe parfois, chez des postlarves de taille voisine, une dissymétrie entre les hémicorps droit et gauche résultant de l'absence de certaines protonéphridies haptoriales.

À ce stade, il existe dans les collecteurs des flamulae collectoris mais leur nombre et leur localisation n'ont pas été précisés.

Pendant le développement ultérieur, le nombre de protonéphridies continue à augmenter, et il est difficile d'en

Fig. 9. Schéma de la disposition du système osmorégulateur chez une postlarve du *Diplectanum aequans* à 19 paires de protonéphridies (d'après Oliver 1987, modifié).



connaître le nombre exact chez les adultes. Chez ces derniers, on observe un nombre important de protonéphridies pharyngiennes et surtout pleurales. L'anse céphalique du collecteur antérieur persiste chez les individus adultes.

La transformation morphologique du *Diplectanum aequans* a une allure allométrique caractérisée d'abord par le développement du hapteur, et ensuite par un fort allongement du corps dans la région postpharyngienne. Cette croissance allométrique se reflète dans la complication du système osmorégulateur. On note ainsi une multiplication des protonéphridies haptoriales pendant la métamorphose du hapteur, puis une forte augmentation des protonéphridies pleurales lors du développement du système digestif et du système génital. Le sous-ensemble antérieur reste relativement plus simple. La multiplication des protonéphridies céphaliques est tardive, celle des protonéphridies pharyngiennes, plus marquée, paraît liée à la croissance de la région immédiatement postpharyngienne, là où apparaîtra l'appareil copulateur.

Oliver (1987) compte, chez une postlarve de 450 μm de longueur d'un autre Diplectanidae, le *Furnestinia echeneis*, neuf protonéphridies par hémicorps. Ce système osmorégulateur, caractérisé par le développement du sous-ensemble postérieur, peut être représenté par la formule 2[(2 + 1) + (2 + 4)].

Chez une postlarve semblable du *Diplectanum aequans*, on remarque une disposition différente : cette dernière a un sous-ensemble antérieur comprenant deux protonéphridies pharyngiennes au lieu de deux protonéphridies céphaliques. Cela peut traduire, entre les deux espèces, un développement légèrement différent de la région antérieure du corps.

Le développement postlarvaire du système osmorégulateur de l'*Acanthocotyle lobianchi*, tel que l'a décrit Kearn (1967), soulève un certain nombre de questions. À l'éclosion, l'oncomiracidium n'est pas cilié. Le système osmorégulateur (fig. 10A) peut être interprété de deux façons : (*i*) soit il manque la protonéphridie pleurale et le système répond à la formule 2[(1 + 2) + (0 + 1)]; (*ii*) soit il manque la protonéphridie haptoriale et la formule représentative est alors 2 [(1 + 2) + (1 + 0)].

Les états ultérieurs sont caractérisés par l'apparition, dans la moitié postérieure du corps, d'une deuxième (fig. 10B) puis d'une troisième protonéphridie (fig. 10C) qui, selon l'interprétation, peuvent être (*i*) soit haptoriales, les formules seraient alors 2[(1 + 2) + (0 + 2)] et 2[(1 + 2) + (0 + 3)]; (*ii*) soit pleurales, les formules seraient alors 2[(1 + 2) + (2 + 0)] et 2[(2 + 2) + (3 + 0)].

Au stade suivant (fig. 10D), marqué par l'apparition des épines dans le prohapteur, on aurait (*i*) soit quatre protonéphridies haptoriales et une ou deux protonéphridies pleurales; (*ii*) soit deux groupes de protonéphridies pleurales : un groupe postérieur (quatre protonéphridies) au niveau du prohapteur, et un autre (une ou deux protonéphridies) dans le corps proprement dit.

Dans la suite, Kearn a noté une multiplication de toutes les protonéphridies (fig. 10E).

Ce développement du système osmorégulateur, un des premiers décrit, ne ressemble pas à celui du *Diplectanum aequans* que nous jugeons plus caractéristique des Monopisthocotylea. En particulier, le débouché direct des protonéphridies dans ce qui est représenté comme le collecteur postérieur nous paraît extraordinaire.

Si l'on admet que chez l'*Acanthocotyle lobianchi*, le collecteur postérieur, constant chez tous les monogènes que nous avons étudiés au cours de nos recherches antérieures, n'a pas été observé, quelle explication peut-on proposer?

Dans la première hypothèse (*i*), il existerait des protonéphridies haptoriales et pleurales, mais ces dernières, absentes chez l'oncomiracidium, apparaîtraient plus tardivement.

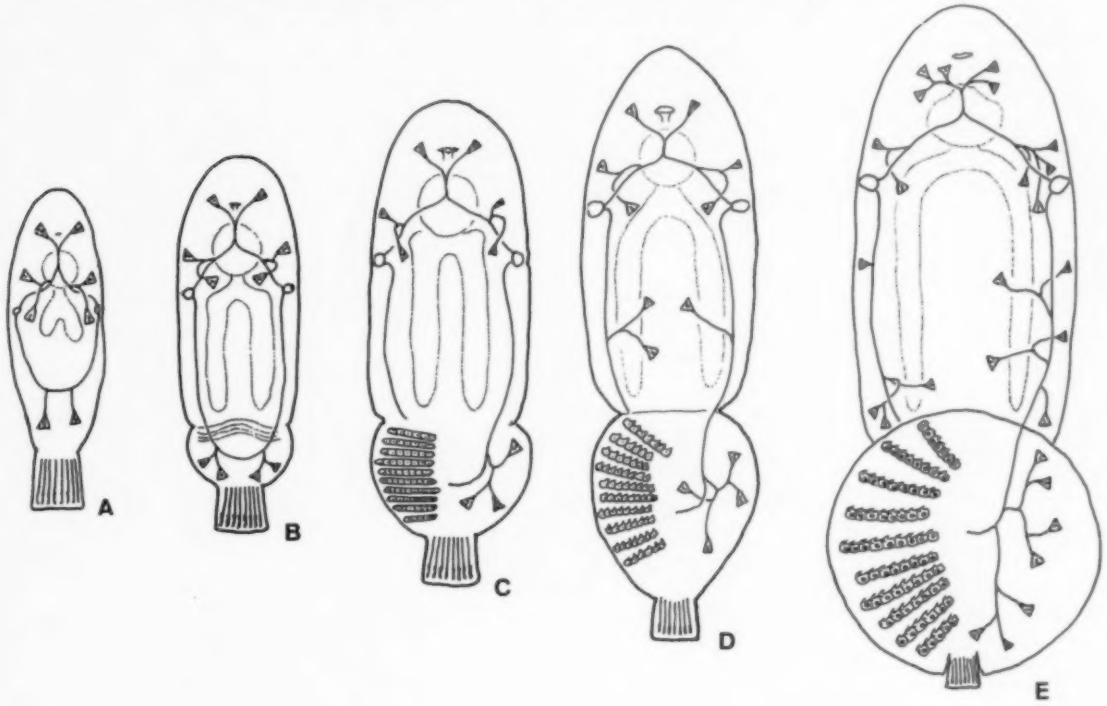
Dans la deuxième hypothèse (*ii*), le hapteur, conservant sa taille larvaire, n'aurait pas de protonéphridie haptoriale (ou une seule non observée). Le prohapteur se formant dans la partie postérieure du corps, les protonéphridies observées à son niveau seraient des protonéphridies pleurales. Elles apparaîtraient ainsi, comme le prohapteur, avant celles de la région du corps où se formeront les organes génitaux.

Le développement du système osmorégulateur des Acanthocotylidae est donc à revoir pour trancher entre les deux hypothèses.

Polyopisthocotylea

Les données sont un peu plus importantes. Ktari (1971*b*) a suivi le développement postlarvaire du système osmorégulateur

Fig. 10. Schéma du développement postlarvaire du système osmorégulateur de l'*Acanthocotyle lobianchi*. (A) Oncomiracidium. (B) Postlarve avec les rides marquant la position du futur pseudo-hapteur. (C) Postlarve avec l'ébauche du pseudo-hapteur. (D) Stade postlarvaire après la formation des sclérites du pseudo-hapteur. (E) Stade postlarvaire montrant la multiplication des protonéphridies. (D'après Kearn 1967, modifié.)



de deux espèces appartenant à deux familles différentes.

Chez le *Pagellicotyle mormyri* le système osmorégulateur de l'oncomiracidium répond au plan fondamental (état Po1), c'est-à-dire 2[(1 + 1) + (1 + 1)], avec les canalicules céphaliques bien séparés (fig. 1).

Comme chez tous les Microcotylidae connus, après la fixation de l'oncomiracidium sur la branchie du poisson hôte, l'ontogenèse postlarvaire débute par la perte des cellules ciliées puis par la formation, dans la partie postérieure du hapteur, de la première paire de pinces (au niveau des crochetons latéraux 1) (fig. 11 A). Le système osmorégulateur a une disposition semblable à celui de l'oncomiracidium avec, comme formule : 2[(1 + 1) + (1 + 1)]. De chaque côté, les pinces suivantes apparaissent successivement, d'arrière en avant, au niveau des crochetons latéraux 2 (stade II : deux paires de pinces) puis 3 (stade III : trois paires de pinces) et 4 (stade IV : quatre paires de pinces). Au stade II le système osmorégulateur n'a pas évolué (fig. 11B).

Au stade III, apparaît une deuxième protonéphridie haptoriale (fig. 11C). Le système peut alors être représenté par la formule 2[(1 + 1) + (1 + 2)].

Au stade IV, on note la présence d'une troisième protonéphridie haptoriale (fig. 12A). Cette disposition répond à la formule 2[(1 + 1) + (1 + 3)].

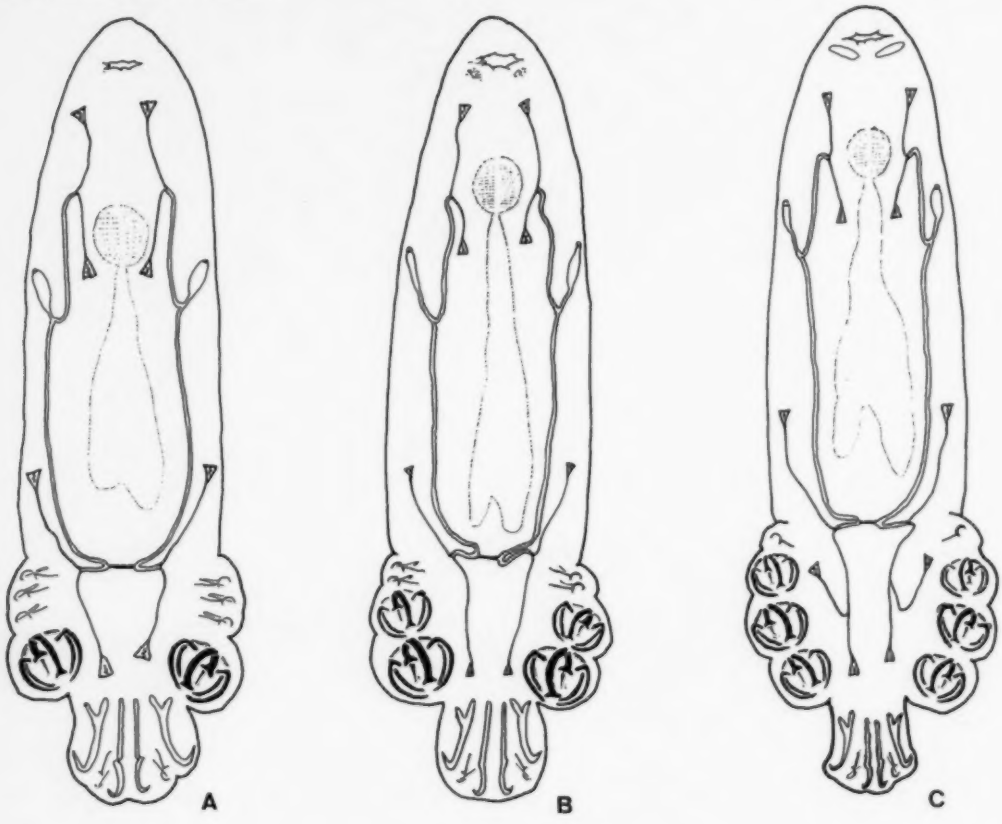
Au stade VI, la languette postérieure portant les deux hamuli, les deux crochetons postéro-latéraux et les deux crochets postérieurs disparaît. Cependant, dans quelques cas, cette languette persiste jusqu'au stade à neuf paires de pinces, mais jamais au delà. Au stade VI (fig. 12B), le sous-ensemble antérieur n'a pas varié mais, dans le sous-ensemble postérieur, est apparu une quatrième protonéphridie haptoriale et une deuxième protonéphridie pleurale. Le système osmorégulateur peut alors être schématisé par la formule 2[(1 + 1) + (2 + 4)].

Lorsque la postlarve possède un hapteur à sept paires de pinces, on observe, à l'avant, une deuxième protonéphridie céphalique et, dans le sous-ensemble postérieur, quatre protonéphridies pleurales et six protonéphridies haptoriales (fig. 12C). Cette disposition peut se traduire par la formule 2[(2 + 1) + (4 + 6)].

La suite du développement n'a pas été suivie en détail. La présence d'une deuxième protonéphridie pharyngienne a été notée seulement au stade XX ou XXI (20 ou 21 paires de pinces). Chez cette postlarve, on compte alors quatre protonéphridies céphaliques et de nombreuses protonéphridies pleurales et haptoriales.

Il existe certainement des variations dans l'ontogenèse du système osmorégulateur telle que nous venons de la décrire. Ainsi, chez une postlarve au stade III du *Microcotyle pomatomi* Goto, 1899, nous avons observé de nombreuses protonéphridies haptoriales, dont deux dans la languette postérieure.

Fig. 11. Schéma du développement postlarvaire du système osmorégulateur du *Pagellicotyle mormyri*. (A) Stade I (une paire de pinces). (B) Stade II (deux paires de pinces). (C) Stade III (trois paires de pinces). (D'après Ktari 1971*b*, modifié.)



Si le système osmorégulateur chez l'oncomiracidium du *Gotocotyla acanthura* Parona et Perugia, 1896 parasite du *Pomatomus saltatrix* répond au plan fondamental 2[(1 + 1) + (1 + 1)], donc ressemble à celui du *Pagellicotyle mormyri*, le développement postlarvaire est différent. En effet, on n'a pas observé la formation de nouvelles protonéphridies haptoriales, et de ce fait, le canalicule haptorial reste très court. Le canal transverse reliant les collecteurs postérieurs droit et gauche persiste dans le hapteur au niveau de la première paire de pinces (la plus postérieure). C'est surtout à partir des protonéphridies pleurales que le système osmorégulateur va se compliquer. Dans chaque hémicorps, apparaît une deuxième protonéphridie pleurale. Ces deux protonéphridies sont chacune à l'origine d'un groupe de protonéphridies pleurales, un au niveau du hapteur (groupe pleuro-haptorial) et l'autre au niveau du corps (groupe eupleural). Ainsi chez une postlarve au stade à cinq paires de pinces (fig. 13), il existe 12 protonéphridies pleurales (7 pleuro-haptoriales et cinq eupleurales). Les canalicules des protonéphridies du groupe pleuro-haptorial forment, en s'unissant, un canal ascendant; les canalicules des protonéphridies eupleurales s'unissent en un canal descendant. Ces deux canaux se rejoignent en avant de la pince la plus antérieure et forment le canal pleural descendant. C'est ce canal qui, à l'extrémité postérieure du hapteur, s'unit au canalicule haptorial pour donner le collecteur postérieur ascendant.

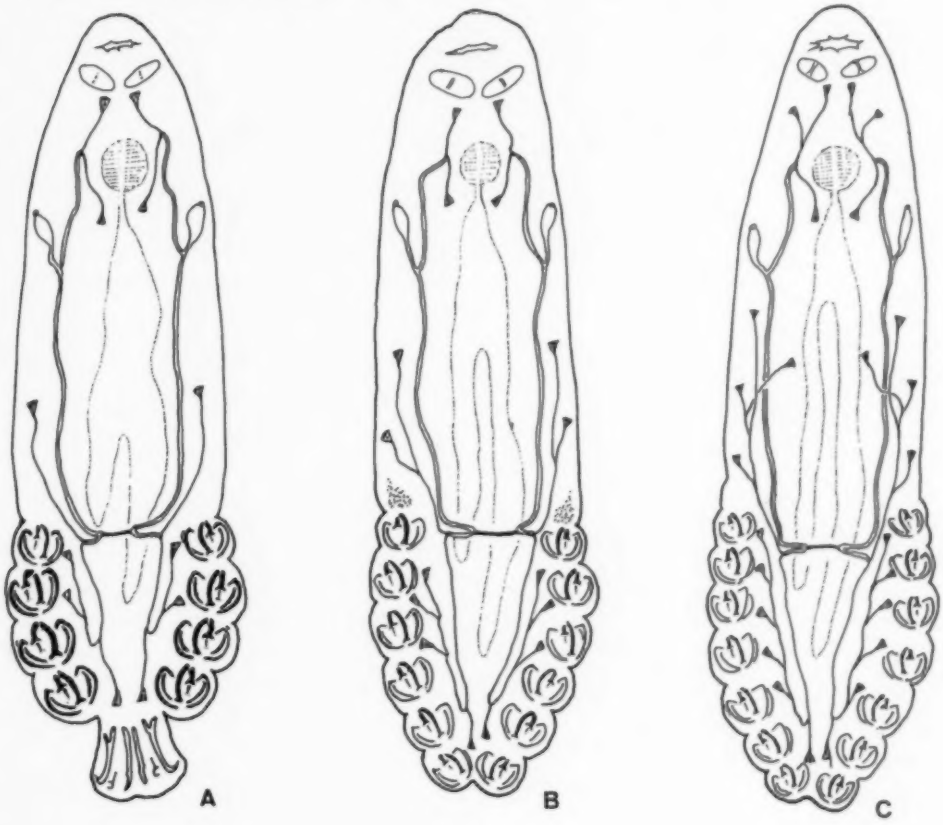
De chaque côté, on peut alors observer (*i*) dans le corps, deux conduits presque parallèles : le canal eupleural descendant et le collecteur postérieur ascendant; (*ii*) dans le hapteur, trois conduits : un descendant (le canal pleural) et deux ascendants (le canal pleuro-haptorial et le collecteur postérieur).

Dans cette postlarve, le sous-ensemble antérieur est peu développé. On a noté seulement trois protonéphridies céphaliques et une pharyngienne. Au stade à six paires de pinces, on peut représenter le système osmorégulateur par la formule 2[(3 + 1) + (13 + 1)].

Notons les limites de la représentation d'une disposition anatomique par une formule. Nous ne pouvons indiquer, dans celle schématisant le système osmorégulateur du *Gotocotyla acanthura*, la distinction, au sein du groupe pleural, des deux sous-groupes pleuro-haptorial et eupleural.

Ktari (1971*b*) a signalé, sur les branchies du *Engraulis encrasicolus*, de grandes postlarves ayant commencé une phase de croissance corporelle, puisqu'elles avaient atteint

Fig. 12. Schéma de la suite du développement postlarvaire du système osmorégulateur du *Pagellicotyle mormyri*. (A) Stade IV (quatre paires de pinces). (B) Stade VI (six paires de pinces). (C) Stade VII (sept paires de pinces). (D'après Ktari 1971*b*, modifié.)



450 à 500 μm de longueur. Ces postlarves se caractérisent par le développement, à l'avant, de deux grosses masses glandulaires et, à l'arrière, d'un hapteur qui conserve l'armature larvaire sans pinces. D'après la morphologie des crochets postérieurs et des hamuli de ce hapteur, on peut rattacher cette postlarve aux Gastrocotylidae (probablement *Gotocotyla acanthura*).

Les observations montrent que le système osmorégulateur de ces postlarves a atteint une complication comparable à celle atteinte au stade VI du développement normal, tel qu'il est décrit ci-dessus (fig. 14).

Le système est en effet composé d'une protonéphridie haptoriale, de deux groupes de protonéphridies pleurales (trois pleuro-haptoriales et trois eupleurales) d'une protonéphridie pharyngienne et de trois protonéphridies céphaliques. Cette disposition peut être représentée par la formule $2[(3 + 1) + (6 + 1)]$. En rapport avec la taille de ces postlarves, le trajet du canal pleural est encore court. La disposition du système osmorégulateur de ces postlarves de Gastrocotylidae confirme ce qui a été observé dans le développement de ce système chez le *Gotocotyla acanthura*.

Ainsi, chez deux Polyopisthocotylea appartenant à deux familles (Microcotylidae et Gastrocotylidae) d'abord fusionnées puis souvent rapprochées, le développement postlarvaire du système osmorégulateur est nettement différent. Dans ces deux familles, l'ontogenèse du système osmorégulateur reflète celle du développement allométrique des individus. Mais si la croissance morphologique du hapteur est comparable, la partie haptoriale du système osmorégulateur a une origine ontogénique différente. Chez le *Pagellicotyle mormyri*, cette dernière partie est formée de protonéphridies dérivant de la protonéphridie haptoriale, alors que chez le *Gotocotyla acanthura*, elle est composée de protonéphridies d'origine pleurale.

Nous n'avons pas actuellement, au sein de ces familles, d'autres exemples de développement du système osmorégulateur pour savoir si cette différence représente un caractère général.

Discussion

La composition et la disposition du système osmorégulateur chez l'oncomiracidium des Monogènes répond à un plan fondamental que l'on retrouve, presque identique, chez les Monopisthocotylea et les Polyopisthocotylea. La différence principale entre les deux sous-classes porte sur

Fig. 13. Schéma de la disposition du système osmorégulateur chez une postlarve du *Gotocotyla acanthura* de Pomatomus saltatrix au stade VII (sept paires de pinces). (D'après Ktari 1971*b*, modifié.)

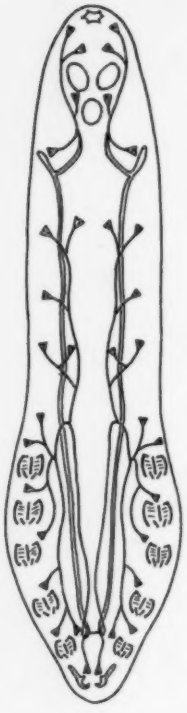
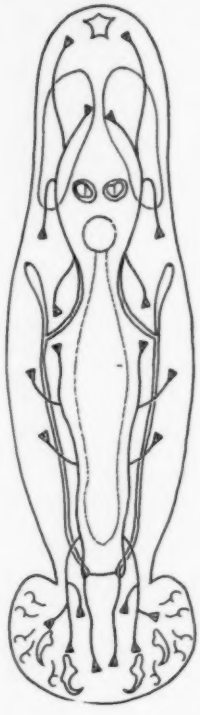


Fig. 14. Schéma de la disposition du système osmorégulateur d'une postlarve de Gastrocotylidae (probablement le *Gotocotyla acanthura*) récoltée chez l'*Engraulis encrasicolus*. (D'après Ktari 1971*b*, modifié.)



le trajet du canalicule des deux protonéphridies céphaliques. Ces canalicules sont décrits comme unis sur le plan médio-sagittal dans la région prépharyngienne chez les Monopisthocotylea et séparés chez les Polyopisthocotylea.

On a cependant observé des canalicules céphaliques parallèles et contigus chez l'oncomiracidium de certains Capsalidae (Monopisthocotylea). Par contre, on a décrit des canalicules céphaliques unis chez l'oncomiracidium de quelques Hexabothriidae (Polyopisthocotylea). Cette disposition reste cependant à préciser, car les canalicules céphaliques ne seraient pas séparés mais étroitement parallèles chez plusieurs oncomiracidia (Maillard 1966; Whittington 1987).

Ainsi, la disposition du système osmorégulateur marque, avec d'autres caractères, l'originalité des Capsalidae parmi les Monopisthocotylea et celle des Hexabothriidae parmi les Polyopisthocotylea.

Nous n'avons pas d'informations sur le système osmorégulateur de l'oncomiracidium des Polystomatidae. Étant donné la position controversée de cette famille dans la systématique des Monogenea, cette absence est particulièrement regrettable.

À partir des connaissances acquises, on peut remarquer que les cinq états (Mo1–Mo5) que nous avons distingués dans le plan du système osmorégulateur de l'oncomiracidium des Monopisthocotylea correspondent en fait à des étapes du développement de ce système, tel que nous l'avons observé chez le *Diplectanum aequans* :

État chez l'oncomiracidium	Étape du développement
Mo1 = 2[(1 + 1) + (1 + 1)]	2[(1 + 1) + (1 + 1)]
Mo2 = 2[(1 + 2) + (1 + 1)]	2[(1 + 2) + (1 + 1)]
Mo3 = 2[(1 + 2) + (1 + 2)]	2[(1 + 2) + (1 + 2)]
Mo4 = 2[(2 + 2) + (1 + 2)]	
Mo5 = 2[(2 + 2) + (1 + 4)]	2[(1 + 2) + (2 + 4)]

On peut en conclure que les oncomiracidia naissent à des stades plus ou moins avancés de leur ontogenèse, les plus avancés étant ceux des Capsalidae. Il semble exister, parmi les représentants de cette famille, un rapport entre l'état du système osmorégulateur et le développement morphologique du hapteur. Ainsi chez le *Capsala onchidiocotyle*, où le système osmorégulateur de l'oncomiracidium est à l'état Mo4, 2[(2 + 2) + (1 + 2)], le hapteur présente 14 crochetons et seulement l'ébauche des deux sclérites médians antérieurs (Euzet 1958). Par contre, chez le *Benedenia monticellii*, où l'oncomiracidium possède, en plus des 14 crochetons, les sclérites médians antérieurs et l'ébauche des deux crochets, le système osmorégulateur est à l'état Mo5, c'est-à-dire à un état ontogénique plus

avancé, puisqu'on peut le schématiser par la formule 2[(1 + 2) +(1 + 4)] ou même 2[(1 + 2) + (3 + 4)].

Parmi les 19 espèces de Monopisthocotylea dont on connaît avec précision le système osmorégulateur de l'oncomiracidium, il ne paraît pas y avoir un état dominant, puisque celles-ci se répartissent de la manière suivante : Mo1 = 6, Mo2 = 5, Mo3 = 2, Mo4 = 1 et Mo5 = 5.

Cette répartition peut être présentée comme un nouvel argument en faveur du polyphylétisme des Monopisthocotylea, idée déjà avancée, basée sur les résultats de l'étude de la chétotaxie et de la couverture ciliée larvaire (Lambert 1980), de l'anatomie de l'appareil génital et de l'ultrastructure du spermatozoïde (Justine 1991).

Polyopisthocotylea

Le système osmorégulateur de la majorité (28/42) des oncomiracidia connus est à l'état Po1. Quelques larves, cependant, naissent avec un système un peu plus compliqué, mais il ne semble pas que cet état, que l'on peut qualifier d'ontogéniquement avancé, soit directement en rapport avec le développement morpho-anatomique de l'oncomiracidium. Ainsi six oncomiracidia ont, à la naissance, le hapteur avec la première paire de pinces. Il s'agit de : *Polylabris tubicirrus*, *Pagellicotyle mormyri*, *Microcotyle mugilis*, *Diplozoon paradoxum*, *D. gracile* et *D. nipponicum*.

Chez le *Polylabris tubicirrus* et le *Pagellicotyle mormyri*, la disposition du système osmorégulateur correspond au plan fondamental Po1. Le système osmorégulateur de l'oncomiracidium du *Microcotyle mugilis* est à l'état Po3. Il est caractérisé par une multiplication des protonéphridies haptoriales et pleurales et peut être représenté par les formules 2[(1 + 1) + (1 + 4)], 2[(1 + 1) + (2 + 4)] et 2[(1 + 1) + (3 + 4)].

Euzet et Combes (1969) estiment que cette multiplication des protonéphridies du sous-ensemble postérieur traduit, chez cet oncomiracidium, un stade ontogéniquement avancé. On peut alors poser la question suivante : pour quelle raison le système osmorégulateur est-il au plan fondamental Po1 chez l'oncomiracidium du *Polylabris tubicirrus* et du *Pagellicotyle mormyri*?

Chez le *Diplozoon gracile*, le *D. paradoxum* et le *D. nipponicum*, le système osmorégulateur est original (état Po5) avec dédoublement des quatre protonéphridies du plan fondamental. Cela pourrait être considéré comme un stade avancé si nous connaissions le développement ultérieur du système.

Le petit nombre d'oncomiracidia possédant un système osmorégulateur légèrement différent du plan fondamental peut être le reflet d'une origine monophylétique des Polyopisthocotylea, en mettant à part les Hexabothriidae et les Polystomatidae qui nécessitent de nouvelles recherches.

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Some pathways and factors of monogenean microevolution[1]

A.V. Gusev

Abstract: This paper discusses probable causes for the origin and evolution of groups of monogenean congeneric sibling species infecting one fish host species, with special reference to freshwater fishes. These causes comprise strict specificity, topological differentiation of parasite micropopulations in microniches (microhabitats), ecological and geographic isolation of various parasite and host populations, paleogeographic or geomorphologic (but *not* paleogeologic) changes during the late Tertiary and Quaternary periods, with alternating marine transgressions and regressions acting upon continents which, after the Miocene, have been less affected by continental drift, orogenesis, and climatic fluctuations. Co-evolving with their hosts, Monogenea usually diverge faster than their hosts.

Résumé : Cet article expose les causes probables de l'origine et de l'évolution des groupes d'espèces monogéniennes jumelles appartenant à un même genre infectant une espèce hôte de poisson, en particulier les poissons d'eau douce. Ces causes englobent la spécificité stricte, la différenciation topologique des micropopulations parasites en microniches (microhabitats), l'isolement écologique et géographique de divers parasites et de leurs populations hôtes, les changements paléogéographiques et géomorphologiques (mais *pas* paléogéologiques) qui se sont produits à la fin du Tertiaire et durant le Quaternaire, périodes marquées par des avancées et des reculs successifs des océans sur les continents qui, après le Miocène, ont été moins profondément touchés par la dérive des continents, l'orogénèse et les fluctuations climatiques. Coévoluant avec leurs hôtes, les Monogéniens divergent habituellement plus rapidement que leurs hôtes.
[Traduit par la Rédaction]

During the 1970's some French authors (Euzet 1972; Oliver 1974, 1976; Ktari 1975) turned their attention to the phenomenon of congeneric doublets of monogenean species occurring on the same host species and the same host individual. Their work, however, overlooked the fact that the same phenomenon, embracing not only congeneric doublets but even multiplets, had been discussed earlier (e.g., Dogiel 1949, 1962; Gusev 1955). This omission is attributable to the language barrier rendering the work of Russian scientists inaccessible to their western colleagues. It is the aim of this paper to breach this barrier and to bring the work of the Russian specialists to the attention of these colleagues.

The occurrence on a single fish host species in one geographic region or one catchment area of not only doublets, but even of multiplets, i.e., groups comprising up to 10 or more congeneric species (species clusters), is one of the fairly commonly occurring features of monogenean communities. The genus *Dactylogyrus* (Dactylogyridae, common gill parasites of Cyprinidae) provides several examples of such occurrence. Of its 13 species occurring on Palearctic roach (*Rutilus rutilus*) and its subspecies, 7 are specific exclusively to this host. These seven represent two intrageneric morphologic (perhaps also genetic) groups. Of 11 species of *Dactylogyrus* found on goldfish, *Carassius auratus*, 7 belong to the *anchoratus-wegeneri* morphologic group and 3 to the *vastator* group; the 11th does not belong to either. On rudd, *Scardinius erythrophthalmus*, two of the three species are closely related, the position of the third is unclear. On nase, *Chondrostoma nasus*, three of six species belong to the same group, the remainder to different other groups. On Amurian steed, *Hemibarbus labeo*, and spotted steed, *H. maculatus*, there are five related species of *Dactylogyrus* and four equally closely "twinned" species of *Ancyrocephalus sensu lato* (Ancyrocephalidae, Ancyrocephalinae). Three species of a single species group of *Silurodiscoides* (Ancyrocephalidae, Ancylodiscoidinae) parasitize European wels, *Silurus glanis*, while 19 related species of another morphologic group live on Amurian namazu, *Parasilurus asotus*. There are eight species of *Anacanthorus* (Ancyrocephalidae) on the Amazonian red piranha, *Pygocentrus nattereri*, and two twin species of *Dactylogyrus* each on tench, *Tinca tinca*, and ruffe, *Gymnocephalus cernuus*.

What are the reasons that have lead to the emergence of such putatively sympatric species (monoxenic, according to Sandground 1929)? This luxuriant speciation might have been prompted by the following factors

1. Strict specificity of most monogeneans. Specificity, i.e., adaptation to a particular host species, on the one hand restricts the evolution of the parasite by hampering its

Received November 23, 1993. Accepted December 15, 1994.
J12517

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[1] Paper first presented at the Second International Symposium on Monogenea, 5–8 July 1991, Montpellier, France.

transfer to, and divergence on, other host species; on the other hand, it opens new evolutionary pathways, parallel to those of the host and, on the host, by facilitating its divergence into different micropopulations inhabiting different microniches (microhabitats of Rohde 1977, 1982). There are many examples of such speciation of monogeneans in parallel with their hosts. Among them are the Indian species *Dactylogyrus lohanii* Gusev, 1976 and *D. chitravanshii* Gusev, 1976; *D. barbi* Gusev, 1976; *D. dubii* Gusev, 1976; and *D. tori* Gusev, 1976; their African and Iraqi relatives *D. ruahae* Paperna, 1973 and *D. barbioides* Gusev, Ali, Abdul-Ameer, Amin and Molnar, 1993, all from different species of *Barbus* (Cyprinidae); and the Indian *Bifurcohaptor indicus* Jain, 1958 and *B. giganticus* Jain, 1958 (Ancyrocephalidae, Ancylodiscoidinae) on different species of *Mystus* (Bagridae). The same is true of several species of *Dactylogyrus* living on Amurian *Hemiculter leucisculus* and *H. eigenmanni* (Cyprinidae, Cultrinae), as well as other groups of related species on different Gobioninae in the Amur River (Gusev 1955) and a group of Palearctic parasites on different Percidae, comprising *Ancyrocephalus paradoxus* Creplin, 1859; *A. percae* Ergens, 1966 and *A. gussevi* Dontsov, 1972. The examples can be multiplied.

2. Topologic differentiation of the parasite's micropopulations in different microniches (microhabitats). The pairs of such species as *Dactylogyroides macracanthus* (Tripathi, 1939) and *D. longicirrus* (Tripathi, 1939) (Dactylogyridae), parasites of *Puntius stigma*; the Black Sea species pair *Diplectanum aculeatum* Parona and Perugia, 1889 and *D. similis* Bykhovsky, 1957, both on *Corvina nigra*, or *Lamellodiscus elegans* Bykhovsky, 1957 and *L. fraternus* Bykhovsky, 1957, both on *Sargus annularis*, can be viewed (as they were by Bykhovsky 1957) as resulting from topologic separation of the monogenean micropopulations. While one member of each of these three pairs generally occurs close to the tips of the gill filaments of its host, the other is found closer to the base of the filaments. According to Paperna (1979) similar differences in microhabitat on the gills characterize the distribution of several species of *Cichlidogyrus* (Ancyrocephalidae). Dogiel (1949) also commented on the widespread occurrence of topologic differentiation of species. Pairs of ecologic forms with right-sided and left-sided clamps present in marine Oligonchoinea: *Gastrocotyle trachuri* Beneden and Hesse, 1863; *Axine belones* Abildgaard, 1794 and *Pseudaxine trachuri* Parona and Perugia, 1890 (see Llewellyn 1956, 1957) are probably on their way to divergence. Scores of other species of higher Monogenea Oligonchoinea also show evidence of similar ecologic and morphologic polymorphism (Lebedev 1986).

3. Ecologic and geographic isolation of various parasite and host populations. This is the common third factor favouring speciation of monogeneans, as well as of other parasites. This factor might be responsible for some differences in species composition within the genus *Silurodiscoides* parasitizing *Parasilurus asotus* in the Amur, as well as in Chinese and Japanese rivers (Gusev and Strelkov 1960; Yu. A. Strelkov, unpublished data). Of 19 species recorded on this fish, only 5 are known in Japan; 2 of these are exclusive to Japan, while 12 others are exclusive to the Asian continent. A good example of the influence of geographic factors is presented by the existence of two closely related species, *Dactylogyrus haplogonus* Bykhovsky, 1933 and *D. haplogonoides* Gusev, 1966, parasitic on vimba, *Vimba vimba*, the former in the rivers of the Caspian Sea basin, the latter in those of the Black Sea basin. The split of their ancestor probably occurred after the disintegration of Pontocaspia, i.e., not more than 1.5 millennia ago (Gusev 1966). In this instance the speciation of the host also played a part, since it also evolved into two distinct subspecies. A similar mechanism appears to have been operative in the speciation of *Dactylogyrus crucifer* Wagener, 1857 and *D. turaliensis* Aligazhdiev, Gusev and Kazieva, 1984 from the Palearctic *Rutilus rutilus*. Differences between forms of the polymorphic species *Dactylogyroides macracanthus* (Tripathi, 1939) from India and Sri Lanka could have resulted from differentiation of isolated populations, or by evolution in parallel with that of the host, different species of Barbus. The same can be said about the closely related species *Dactylogyrus soni* (*nomen novum* for preoccupied *D. gobii* Tripathi, 1939) from India and *D. jubbstrema* Price, Korach and McPott, 1969 from South Africa, both parasites of *Glossogobius giuris*.

4. The fourth factor instrumental in monogenean evolution is paleogeographic, related to geomorphologic processes. It determines all other factors that may be considered with reference to the remote past.

The present-day monogenean fauna, as well as the freshwater fish fauna, might have had its origins in the Middle and Upper Tertiary (Yakovlev 1964; Tsepkin 1967; Gusev 1976, 1978). Apart from the continuous orogenic processes and their attendant effects on the river systems, there had been three or four global scale changes in the sea level and climate at the end of the Tertiary and during the Quaternary period (Lindberg 1955, 1972). The traces of these changes have remained in the form of continental terraces and submerged canyons extending for long distances from the mouths of many large rivers over the continental shelves. The greatest rise in sea level during the high level periods was up to 180–200 m above its present level. Sea water, an insurmountable barrier for typically freshwater organisms, flooded the coastal plains. The aquatic fauna of these plains either perished or was displaced upstream into rivers, their tributaries and lakes of higher altitudes, such as Lake Biwa on the Japanese island of Honshu. The changes in sea levels began with catastrophic rapidity, taking apparently not more than several decades, but the periods of alternating low and high levels extended over millennia. During those marine transgressions populations of fish and their parasites could have become isolated from one another in various tributaries and undergone there independent changes culminating in ecologic and morphologic divergence.

As the result of the drop in temperature and locking away of large volumes of water in glacial ice, the sea retreated 200–300 m below its present level, rivers united into huge basins, their faunas mixed and related or conspecific fishes from various tributaries, now included in the same river systems, exchanged their monogeneans. In consequence, individual fish species (subspecies, morphs, races) acquired clusters of divergent parasites. During these periods of regression representatives of faunistic and morphologic complexes of different origin intermingled with one

another. (These phenomena were documented by Nikolsky (1974) and Gusev (1955, 1964), whose views were subsequently elaborated and improved upon by many other Russian ichthyologists and parasitologists, such as V.N. Yakovlev, S.S. Shulman, Z.S. Donets, V.J. Trofimenko, O.N. Pugachov, and others, mainly representatives of V.A. Dogiel's school of ecologic parasitology.) The effect of this process was the formation of a great variety of multiple monogenean species, which we now observe in many regions on the same fish species and individuals. The seeming sympatry of such species appears to be secondary, but many of them are of allopatric origin (Gusev 1978). Only those species that arose as a result of topologic divergence of various micropopulations within microhabitats of a single host can be considered truly sympatric.

During the periods of lowest sea level there existed extensive river systems of the Paleohwang Ho (comprising the Amur, Hwang Ho and Yangtse rivers), the Paleomekong (combining many rivers of Indochina, Malaysia and Indonesia), the Paleocongo (rivers of western Africa and the Gulf of Guinea), the Paleorhine, the Paleodanube, etc. (Lindberg 1972). These basins have broken into their individual components now, and new forms, new taxa evolve independently in each of these components. Should another period of low sea level ensue, they might intermingle again and thus increase the diversity of monogeneans on many fish species. The continental drift and the breakup of Pangea, followed by that of Gondwana and Laurasia, i.e., the paleogeographic factor, exerted its influence predominantly on the formation of the southern freshwater monogenean faunas (African, South American, and South Asian), determining their characteristics and their ancient interrelationships (according to Gusev (1978) some evidence of relationship between South American and South Asian faunas can be found in related taxa). In contrast, the formation of the present northern faunas (Palearctic, Amur-Chinese, and Sonoran) and the microevolution of monogeneans have been determined largely by the succession of alternating marine transgression and regression phases, and, in more recent time, by the presence of the Bering bridge (Gusev 1976, 1978).

In many instances species might have diverged owing to the combination of several factors, all of them fostering disjunction of habitats of individual populations or micropopulations, i.e., their reproductive isolation in various restrictive niches (macro- and micro-habitats of Rohde 1977, 1982) (see also Llewellyn 1956, 1957).

Finally, one other possible mechanism of speciation should be mentioned. I refer to the phenomenon of "organ heterotopy," appearing in various forms, described by Lebedev (1969) in *Pentatres sphyraenae* Euzet and Razarihelisoa, 1959, a higher marine monogenean (Oligonchoinea, Chauhaneidae). Should such change in the arrangement of organs be accompanied by some ecologic or geographic differentiation in parts of a population, these parts may eventually evolve into isolated forms or taxa. I do not know of any example of "organ heterotopy" among lower freshwater monogeneans (Polyonchoinea).

Regrettably, it is impossible to corroborate the propositions outlined above by data derived from molecular biology on monogenean species or their infraspecific groups. I know of no publications on this topic. Karyological investigations are very scanty. Morphology, host ranges, and geographic distribution are the only criteria I could use.

The impact of the factors enumerated above on monogenean speciation may be due to the following features of these helminths. (1) Strict specificity of most species. (2) Their morphological plasticity, when faced with fragmentation of populations or micropopulations into several isolated parts. (3) The existence of one *adaptive barrier* other than the external environment, i.e., the presence of only one host in their simple life cycle (monoxeny of Sandground 1929). (Parasites exhibiting a low degree of specificity and characterized by complex life cycles with changes of hosts (polyxeny) confront more adaptive barriers.) (4) Active selection of suitable host (niche) by their free-swimming larvae. The selective capabilities of monogenean larvae are due to their ability to sense certain pheromones (attractant "parasitaepagones" or repellent "parasitaminones" (Gusev 1978)), present in the mucus of every host species. This statement is based on the results of the experiments conducted by Kearn (1967), Izyumova (1970) and others.

In lieu of a conclusion, I would like to add the following statement. It has been widely held in the past that in the event of co-evolution of a host and its specific parasite, the pace of change of the latter lags behind that of the former (the so-called Rubtsov's Rule (Rubtsov 1939; Dogiel 1962). Indeed, numerous parasites, particularly monogeneans, furnish many examples of the operation of this "rule." A single species, *Dictylobothrium armatum* Leuckart, 1835 (Oligonchoinea, Dictylobothriidae) parasitizes various species of sturgeons (Acipenseridae), fishes of Paleozoic lineage, in several zoogeographic regions and provinces. Different species of pike (Esocidae) harbour *Tetraonchus monenteron* Wagener, 1857 in Europe, in the Amur River, and in North America. Different species and genera of Cyprinidae carry *Dactylogyrus sphyrna* Linstow, 1978. The Palearctic common carp, *Cyprinus carpio*, introduced into North America and the Amurian carp, *C. haematopterus*, carry *Dactylogyrus extensus* Mueller and Van Cleave, 1932; *D. vastator* Nybelin, 1924; *D. achmerovi* Gusev, 1955 and *D. minutus* Kulwiec, 1927. The crucian carp, *Carassius carassius*, and the goldfish, *C. auratus*, harbour 10 species of *Dactylogyrus* in the same region.

However, many facts point to a different balance between the rates of evolution of the host and its parasite. This is illustrated by all examples quoted in the first part of this paper. Their numbers could be multiplied, but it is hardly necessary. I will add only that the just-mentioned goldfish harbours in the Far East three species of *Dactylogyrus* absent from other regions. The Amurian carp, *Cyprinus haematopterus*, also carries three species of *Dactylogyrus* absent from Palearctic *C. carpio*. In fact, the correlation between the rates of evolution in the host–parasite system exists in all its possible variants: the parasite may lag behind the host; both components of the system may evolve at the same rate; and the parasite may evolve faster than the host. The last of these variants appears to be the most common in Monogenea (see Gusev 1955).

Acknowledgements

The invitation to contribute to this volume dedicated to Dr. Leo Margolis, a friend and an outstanding scientist, has been a great honour for me. I greatly regret that the limitation of space did not permit me to give a clearer and more detailed account of the postulate that has been comprehensively treated in earlier, mainly Russian, publications. I thank Dr. Z. Kabata for his friendly help in preparing this paper for the press. My thanks are also due to the editor, Dr. J. R. Arthur, and the anonymous referees for valuable critical but constructive comments, which I tried to take into account in writing the final draft of the paper. The writing of this paper was supported by the George Soros International Science Foundation.

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A population-phenetic analysis of morphological polymorphism in *Polystoma integerrimum* (Monogenea)

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Abstract: The analysis of population phenetics to study some of the polymorphic characters in *Polystoma integerrimum* (Froelich, 1791) from two populations in the Moscow and Novgorod regions, Russia, revealed discrete variations of these characters, defined as phenes, which are discrete, usually alternative, simple features reflecting the peculiarities of a given genotype. The studies demonstrate that the frequencies of phenes B1, B2, and B3, characterized by peculiarities of the serrations on the lower basal part of the marginal hooks, define the differences in genetic structure of the populations studied. Besides these structural differences, the two populations of *Polystoma* differ in intrapopulational phenetic diversity, whose index was higher in the Novgorod population. It is suggested that variations in the number of chitinous hooks of the copulatory organ i.e., 5, 6, 7, 9, and 10 hooks, characterize the complex of East-European populations of *P. integerrimum*.

Résumé : L'analyse de la phénétique des populations en vue d'étudier certains des caractères polymorphes du *Polystoma integerrimum* (Froelich, 1791) dans deux populations des régions de Moscou et de Novgorod (Russie) a révélé des variations discrètes de ces caractères, définis comme des phènes, qui sont des traits simples discrets, généralement alternatifs, reflétant les particularités d'un génotype donné. Les études montrent que la fréquence des phènes B1, B2 et B3, caractérisés par des particularités des dentelures de la partie basale inférieure des crochets marginaux, définissent les différenecs de la structure génétique des populations étudiées. Outre ces différences structurales, les deux populations de *Polystoma* diffèrent par la diversité phénétique intrapopulation, dont l'indice était plus élevé chez la population de Novgorod. Nous pensons que les variations du nombre de crochets chitineux de l'organe copulateur (5, 6, 7, 9 et 10 crochets) caractérisent le complexe de populations est-européennes de *P. integerrimum*.
[Traduit par la Rédaction]

Population phenetics, the application of genetic methods and principles to species whose genetic study is difficult or impossible, has developed significantly in the past 20 years. New methodological approaches to the study of natural populations have provided conditions conducive to the appearance of population phenetics as an original research trend (Yablokov and Larina 1985). It aims to reveal and study discrete variations of any feature (e.g., morphological, physiological, biochemical, etc.) that marks genetic peculiarities of different groups within a species (Yablokov and Larina 1985).

Investigation of population phenetics is based on the notion of the phene, an inherited varying character. Phenes are discrete alternative variations of some character or property that are indivisible without loss of property in a large sample of individuals studied. They reflect the genotypic constitution of the individual, whereas frequencies of their occurrence reflect the genetic peculiarities of a group of individuals (Yablokov and Larina 1985). Basic data on studies of population phenetics are contained in several Russian publications, e.g., *Phenetics of Populations* and *Phenetics of Natural Populations*, both edited by A.V. Yablokov (Yablokov 1982, 1988), as well as in R.J. Berry's book *Inheritance and Natural History* (Berry 1977). Most publications in the field of population phenetics are devoted to studying free-living organisms and only in recent years have reports on the population phenetics of species of parasitic helminths begun to appear (Popov and Fortunato 1985; Gichenok and Bartakova 1990; Malashenko 1994).

The population-genetic analysis of morphological polymorphism in Monogenea can considerably enlarge knowledge in the fields of microevolution, phylogeny, and systematics in this group of parasites. Among the indices developed for estimation of polymorphism in living organisms, the

Received April 7, 1994. Accepted August 25, 1994.
J12780

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most informative are those of population variability based on polymorphic characters, as elaborated by Zhivotovsky (1979, 1980, 1982), information on which will be given below. They have been successfully applied to population analysis of polymorphism in the chitinous armament of *Dactylogyrus vastator* Nybelin, 1924 (see Fortunato 1987).

The purpose of our study was to search for polymorphic features in the structure of the chitinous armament of *Polystoma integerrimum* (Froelich, 1791), a parasite of the urinary bladder of frogs (*Rana* spp.), to reveal discrete variations of polymorphic characters that conform to the phene definition, and to study variation in patterns of phene frequency in different parasite populations.

Materials and methods

Specimens of *Polystoma* were collected from frogs (*Rana temporaria*) caught in two localities of the Moscow and Novgorod regions, Russia from May to August 1992. The worms were mounted into polyvinyl following the technique of Gusev (1983). A total of 176 specimens of *P. integerrimum* from 111 host individuals was studied.

Five independent polymorphic characters, namely the number of chitinous hooks of the copulatory organ, the shape of the basal part (bp) of the marginal hook, the serration of the lower margin of the bp, the presence or absence of a notch between the pivot and the lower margin of the bp, and the presence or absence of knob on the middle of the upper edge of the bp, have been subjected to population-phenetic analysis.

For the analysis of frequencies of discrete variations of polymorphic characters, the statistical methods elaborated by Zhivotovsky (1979, 1980, 1982) have been used. For comparison of population samples, the index of population similarity (r) was calculated from the following formula:

$$r = \sqrt{p_1 q_1} + \sqrt{p_2 q_2} + \ldots + \sqrt{p_m q_m}$$

where p_1, p_2, ... p_m and q_1, q_2, ... q_m are frequencies of discrete variations in samples. The index r may range from 0 to 1. To determine if r differs significantly from 1, i.e., whether the populations being compared differ from each other, the identity criterion (I) was calculated according to the following formula:

$$I = \frac{8N_1N_2}{N_1 + N_2}\left(1 - r - \frac{p_o + q_o}{4}\right)$$

where N_1 and N_2 are sizes of samples, p_o is the sum of the frequencies of the discrete variations in sample 1 being absent in sample 2, and q_o is the sum of the frequencies of those sample 2 variations that are absent from sample 1.

The index I has as a distribution similar to chi-square with $m - 1$ degrees of freedom. Therefore, the significance of the difference of r from 1 was estimated by comparison of I with chi-square table values at a significance level of $p = 0.05$.

If r differed significantly from 1 (by criterion I), then the sample error (S_r) was calculated from the following formula:

$$S_r = \frac{1}{2}\sqrt{\frac{1 - q_o - r^2}{N_1} + \frac{1 - p_o - r^2}{N_2}}$$

Table 1. Frequencies for the number of copulatory organ hooks of *Polystoma integerrimum* in two populations.

Locality	No. of hooks				
	n	5–6	7	8	9–10
Moscow region	108	0.102	0.252	0.563	0.083
Novgorod region	42	0.071	0.143	0.643	0.143

The degree of phenetic diversity in populations and within population groups was estimated by the index of intrapopulation diversity (μ), where

$$\mu = \left(\sqrt{p_1} + \sqrt{p_2} + \ldots + \sqrt{p_m}\right)^2$$

and the formula for calculation of the error (S_μ) is

$$S_\mu = \sqrt{\frac{\mu(m - \mu)}{N}}$$

Since n independent characters were analyzed, the mean value μ (the mean number of phenes within population) was calculated as

$$\bar{\mu} = 1/n(\mu_1 + \ldots + \mu_n),\ S_{\bar{\mu}} = 1/n\sqrt{S_1^2 + \ldots + S_n^2}$$

The pattern of interpopulation diversity was estimated from the calculation of the share of rare phenes (h)

$$h = 1 - \mu/m$$

and formula for the calculation of the error (S_h) is

$$S_h = \sqrt{\frac{h(1-h)}{N}}$$

For n independent characters, $h = 1 - \bar{\mu}/\bar{m}$, $S_{\bar{h}} = S_{\bar{\mu}}/\bar{m}$, and $\bar{m} = 1/n(m_1 + m_2 + \ldots + m_n)$, where m_1, m_2, ... m_n are the number of variations found in the corresponding characters.

To estimate the significance of differences between μ and h from different samples, the criterion U was calculated as

$$U = \frac{|\mu_1 - \mu_2|}{\sqrt{S_{\mu_1}^2 + S_{\mu_2}^2}} \quad \text{and} \quad U = \frac{|h_1 - h_2|}{\sqrt{S_{h_1}^2 + S_{h_2}^2}}$$

Values of U were compared with corresponding significance levels according to a normal distribution table.

Results

Analysis of the material collected revealed polymorphic features in the structure of the chitinous parts of the copulatory organ and marginal hooks of *P. integerrimum*. The specimens of *Polystoma* examined varied in chitinous copulatory organ hook number, 5, 6, 7, 8, 9, or 10 hooks being present.

Because few specimens of *Polystoma* in the samples analyzed had 5, 6, 9, or 10 copulatory organ hooks, the specimens with 5 or 6 and 9 or 10 hooks were grouped together to stabilize the estimation of rare variations (Zhivotovsky 1991). For the frequencies of phene occurrence given in Table 1, the values $r = 0.986 \pm 0.015$ and $I =$

3.39 were calculated. Comparison of the I value with the chi-square table value reveals that differences between the populations are not significant ($p > 0.05$) i.e., the frequencies of common phenes are distributed much the same in the populations.

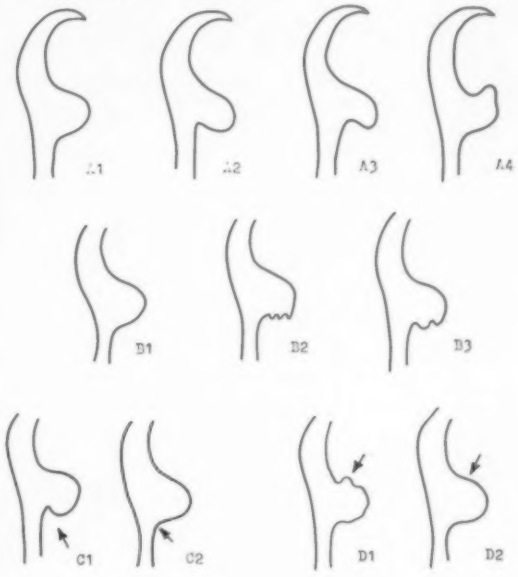
Variability in marginal hook shape was studied in the largest, posterior hook pair, where discrete alternative variations are most pronounced. In the marginal hooks, the shape of the basal part varies. Study of a total of 109 specimens of *P. integerrimum* from the two populations revealed the presence of four polymorphic characters in the structure of the basal part: (A) shape (four variations), (B) serration of the lower margin (three variations), (C) presence or absence of a notch between the pivot and the lower margin, and (D) presence or absence of a knob on the middle of the upper edge (Fig. 1). Thus, for the analysis of the variability in the shape of the basal part of the marginal hook, 11 discrete variations were available.

To ensure that the observed variations are phenes, a comparative analysis was carried out for two samples from a population of *Polystoma* infecting frogs in the Moscow region. The first sample included specimens from 1-year-old and older frogs (the polystomes were of the same age) and the second one consisted of young polystomes collected from frogs that had recently left the pond after their metamorphosis. As a rule, properly isolated phenes retain their stable frequencies for a long period of a population's existence; therefore these samples were compared for the characters A, B, C, and D (Table 2) using the similarity index, for which r is 0.983 ± 0.022, 0.998 ± 0.008, 0.961 ± 0.034, and 0.938 ± 0.042, respectively. Values of I (2.31 for character A, 0.26 for B, 4.98 for C, and 79.0 for D) exceed chi-square table values ($p > 0.05$) for characters C and D. Thus among variations of characters, the A1, A2, A3, B1, and B2 variations can be considered phenes, since their frequencies of occurrence remain stable with time. Consequently they can be used to determine the genetic characteristics of the population of *P. integerrimum* studied. Two characters (A4 and B3) could not be tested, as they were not present in the Moscow population.

The marginal hook phenes isolated above were used to study interpopulational differences (Table 3). The index r calculated for each character, are as follows: 0.936 ± 0.033 and 0.872 ± 0.046 for A and B, respectively (accordingly, $I = 6.66$ for A and $I = 15.7$ for B). The value of I for the character B exceeds the chi-square table value, which is indicative of the presence of significant differences ($p < 0.05$) between the Moscow and Novgorod populations in the frequencies of B1, B2, and B3 phene occurrence.

Using the set of isolated phenes, we also studied the intrapopulational diversity within each population with μ (average phene numbers) estimating the degree of population diversity ($\mu = 2.19 \pm 0.07$ for the Moscow population and $\mu = 3.19 \pm 0.12$ for the Novgorod population) and h characterizing the diversity structure ($h = 0.107 \pm 0.04$ and 0.126 ± 0.032 for the Moscow and Novgorod populations, respectively). Comparison of μ and h between the two populations of *Polystoma* using the U parameter ($U = 3.89$ for μ, $U = 0.5$ for h) with the normal distribution allowed us to conclude that there are significant differences between populations ($p < 0.05$) in the degree of diversity, whereas the differences between proportions of rare phenes in each population are not significant ($p > 0.05$), i.e., these populations have a similar structure of phenetic diversity.

Fig. 1. Discrete variations in the structure of the basal part of the marginal hook of *Polystoma integerrimum*. A1–A4, variations in shape of basal part; B1–B3, variations in serration of lower margin; C1–C2, presence or absence of a notch between pivot and lower margin; D1–D2, presence or absence of a knob on the middle of the upper edge.



Discussion

Phenetic analysis of polymorphism in *Polystoma integerrimum* demonstrated the presence of morphological features defined as phenes. Variations of the B character may be considered phenes of population scale, as their frequencies of occurrence characterize each population. In the *Polystoma* population of the Moscow region the phene B2 was most common and the phene B3 was not found, whereas in the Novgorod population specimens with phenes B1 and B3 predominated.

Each population examined can be distinguished from the viewpoint of intrapopulational diversity characteristics. The populations studied have similar character of phenetic diversity, which is indicated by the absence of differences by U criterion between h indices from the two populations. The differences revealed between μ indices define the population differences according to the degree of intrapopulational diversity. At the same time, the values of the μ indices show that the degree of phenetic diversity is higher in the *Polystoma* population from the Novgorod region.

Studies of variability in the number of chitinous hooks of the copulatory organ, a polymorphic character, may be

Table 2. Frequencies of morphological variants of marginal hooks of *Polystoma integerrimum* in two intrapopulational age-groups.

		Variations								
Age	n	A1	A2	A3	B1	B2	C1	C2	D1	D2
<1 year	26	0.77	0.15	0.08	0.23	0.77	0.35	0.65	0.12	0.88
>1 year	49	0.68	0.16	0.16	0.27	0.73	0.12	0.88	—	1.00

Table 3. Frequencies of the phenes for marginal hooks in two populations of *Polystoma integerrimum*.

		Phenes						
Locality	*n*	A1	A2	A3	A4	B1	B2	B3
Moscow region	49	0.68	0.16	0.16	—	0.27	0.73	—
Novgorod region	34	0.53	0.09	0.29	0.09	0.56	0.32	0.12

of value for systematic studies of the genus *Polystoma*. The number of hooks in the copulatory organ is of diagnostic importance, for example, in the *Polystoma* "africanum" group (Saoud 1967). Original data on the hook numbers in the copulatory organ of *P. integerrimum* can be found only in the works of authors who studied polystomes in western Europe (Zeller 1872; Combes 1968). These authors recorded the hook number as invariably eight. However, our analysis shows that this character is variable and that only some 60% of specimens examined from the two populations had a genital armament of eight hooks. Studies on the biogeography of *P. integerrimum* (Combes 1976; Ryzhikov et al. 1980) indicate that the populations of the Moscow and Novgorod regions are situated on the eastern border of the species range. The centre of distribution is apparently located in western Europe. The stability of frequencies of variations in the number of copulatory organ hooks in the two populations studied allows us to consider them phenes of value in the recognition of suprapopulations. It is suggested that the "5, 6, 7, 9, or 10 hook" phenes distinguish the complex of populations of *P. integerrimum* occurring in eastern Europe.

Acknowledgements

These studies were supported by the J. Soros Fund and the Russian Academy of Natural Sciences. We are grateful to Dr. A. Sysoev and Mrs. O. Kukushkina, Institute of Parasitology of the Russian Academy of Sciences, for their help in the final preparation of the manuscript.

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Redescription of *Benedenia epinepheli* (Yamaguti, 1937) Meserve, 1938 (Monogenea: Capsalidae) from cultured and aquarium marine fishes of Japan

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Abstract: The monogenean *Benedenia epinepheli* (Yamaguti, 1937) Meserve, 1938 is redescribed and reported for the first time from the fins, eyes, and body surface of cultured marine fishes of Japan. Because of close similarity in the general morphology of the reproductive system and median sclerites, comparable morphometrics, and the presence of a flap slightly left of the common genital aperture, *Benedeniella congeri* Yamaguti, 1958 (= *Neobenedeniella congeri* (Yamaguti, 1958); *Benedenia congeri* (Yamaguti, 1958)) and *Benedenia* sp. of Tsutsumi and Ito, 1965 are synonimized with this species. Twelve new hosts are presented, bringing the total number of known hosts to 25 (11 Tetraodontiformes, 9 Perciformes, 3 Scorpaeniformes, 1 Pleuronectiformes, and 1 Anguilliformes). The ability of *B. epinepheli* to cause death of cultured fishes during heavy infection, and its low host specificity, ubiquitous distribution in western Japan, and the high susceptibility of marine aquarium fishes are indicative of the parasite's great potential to become a disease problem.

Résumé : Le monogénien *Benedenia epinepheli* (Yamaguti, 1937) Meserve, 1938 fait l'objet d'une nouvelle description, et sa présence est signalée pour la première fois sur les nageoires, les yeux et la surface du corps chez des poissons marins d'élevage au Japon. Étant donné l'étroite similarité observée dans la morphologie générale du système reproducteur et des sclérites médians, la morphométrie comparable et la présence d'un rabat légèrement à gauche du pore génital commun, *Benedeniella congeri* Yamaguti, 1958 (= *Neobenedeniella congeri* (Yamaguti, 1958); *Benedenia congeri* (Yamaguti, 1958)) et *Benedenia* sp. de Tsutsumi et Ito, 1965, sont mis en synonymie avec la présente espèce. Douze nouveaux hôtes sont présentés, ce qui porte à 25 le nombre total d'hôtes connus (11 Tétraodontiformes, 9 Perciformes, 3 Scorpaeniformes, 1 Pleuronectiforme et 1 Anguilliforme). Le fait que *B. epinepheli* puisse causer la mort des poissons d'élevage en cas d'infestation importante, sa faible spécificité quant à l'hôte, son ubiquité dans l'ouest du Japon et la grande vulnérabilité des poissons d'aquarium indiquent que ce parasite pourrait causer de graves problèmes de maladie.

[Traduit par la Rédaction]

Introduction

Since 1989, *Benedenia epinepheli* (Yamaguti, 1937) Meserve, 1938, a capsalid monogenean originally reported from wild Japanese grouper (*Epinephelus akaara*) more than 50 years ago (Yamaguti 1937), has been recovered from the fins, body surface, and eyes of many species of marine cultured and aquarium fishes of Japan. This paper redescribes this monogenean, presenting previously undescribed morphological structures and proposes a new synonymy. Further, information on host specificity, morphological variability of the parasite's haptoral hard parts, geographic distribution, and associated problems is presented.

Received April 19, 1994. Accepted October 6, 1994.
J12781

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Materials and methods

Parasites were obtained from collections of marine cultured and aquarium fishes in Japan examined between October 1989 and January 1993 (see Table 1).

Specimens were fixed in alcohol – formalin – acetic acid (AFA), stained with alum-carmine or Heidenhain's iron hematoxylin, dehydrated through an alcohol series, and mounted in Canada balsam prior to examination. All measurements are given in millimetres as the range followed by the mean ± standard deviation. Illustrations were made with the aid of a camera lucida. Most of the terminology

Table 1. Information on classification of host fish species, collection localities, and dates for *Benedenia epinepheli* (Yamaguti, 1937) from Japanese fishes.

Order, family	Species	Reference[a]	Locality	Collection date[b]
Tetraodontiformes				
Balistidae	*Balistoides conspicillus*	5A	Tokyo	December 1992
Diodontidae	*Diodon holocanthus*	5A	Hyogo	December 1989
	Aluterus monoceros	4C	Nagasaki	
Ostraciidae	*Ostracion cubicus*	3A	Yamaguchi	
		5A	Hyogo	January 1990
	O. immaculatus	5A	Tokyo	January 1993
Tetraodontidae	*Takifugu pardalis*	3A	Yamaguchi	
	T. porphyreus	3A	Yamaguchi	
	T. poecilonotus	3A	Yamaguchi	
	T. niphobles	3A	Yamaguchi	
	T. radiatus	3A	Yamaguchi	
	T. vermicularis	3A	Yamaguchi	
Perciformes				
Cheilodactylidae	*Goniistius zonatus*	3A	Yamaguchi	
Echeneididae	*Echeneis naucrates*	3A	Yamaguchi	
Labridae	*Semicossyphus reticulatus*	3A	Yamaguchi	
Oplegnathidae	*Oplegnathus punctatus*	5A	Hyogo	December 1989
Pomacanthidae	*Chaetodon auriga*	5A	Tokyo	September 1992
Serranidae	*Epinephelus akaara*	1W	Hyogo	
		5C	Fukui	December 1990
	E. moara	3A		
		5C	Wakayama	February 1990
	E. septemfasciatus	5C	Oita	January 1992
	E. suillus	5C	Kagoshima	June 1992
Scorpaeniformes				
Scorpaenidae	*Sebastes schlegeli*	5C	Yamaguchi	September 1992
	Sebasticus marmoratus	5A	Hyogo	October 1989
	Pterois volitans	5A	Tokyo	October 1992
Anguilliformes				
Anguillidae	*Conger myriaster*	2W	Hyogo	
Pleuronectiformes				
Pleuronectidae	*Paralichthys olivaceus*	5C	Shimane	October 1989
		5C	Oita	July 1992
		5C	Ehime	January 1993

[a]References: 1, Yamaguti 1937; 2, Yamaguti 1958; 3, Tsutsumi and Ito 1965; 4, Kamegai et al. 1985; 5, original; A, aquarium; W, wild; C, cultured.
[b]Dates refer to present study.

used for description is based on Whittington and Kearn (1993), while scientific names of fishes basically follow those given by Nakabo (1993).

Results

Benedenia epinepheli (Yamaguti, 1937) Meserve, 1938
Figs. 1–3

Synonyms: Epibdella epinepheli Yamaguti, 1937; *Benedeniella congeri* Yamaguti, 1958; *Neobenedeniella congeri* (Yamaguti, 1958) Yamaguti, 1963; *Benedenia congeri* (Yamaguti, 1958) Byrnes, 1986; *Benedenia* sp. of Tsutsumi and Ito, 1965.

Type host: *Epinephelus akaara*, redspotted grouper.

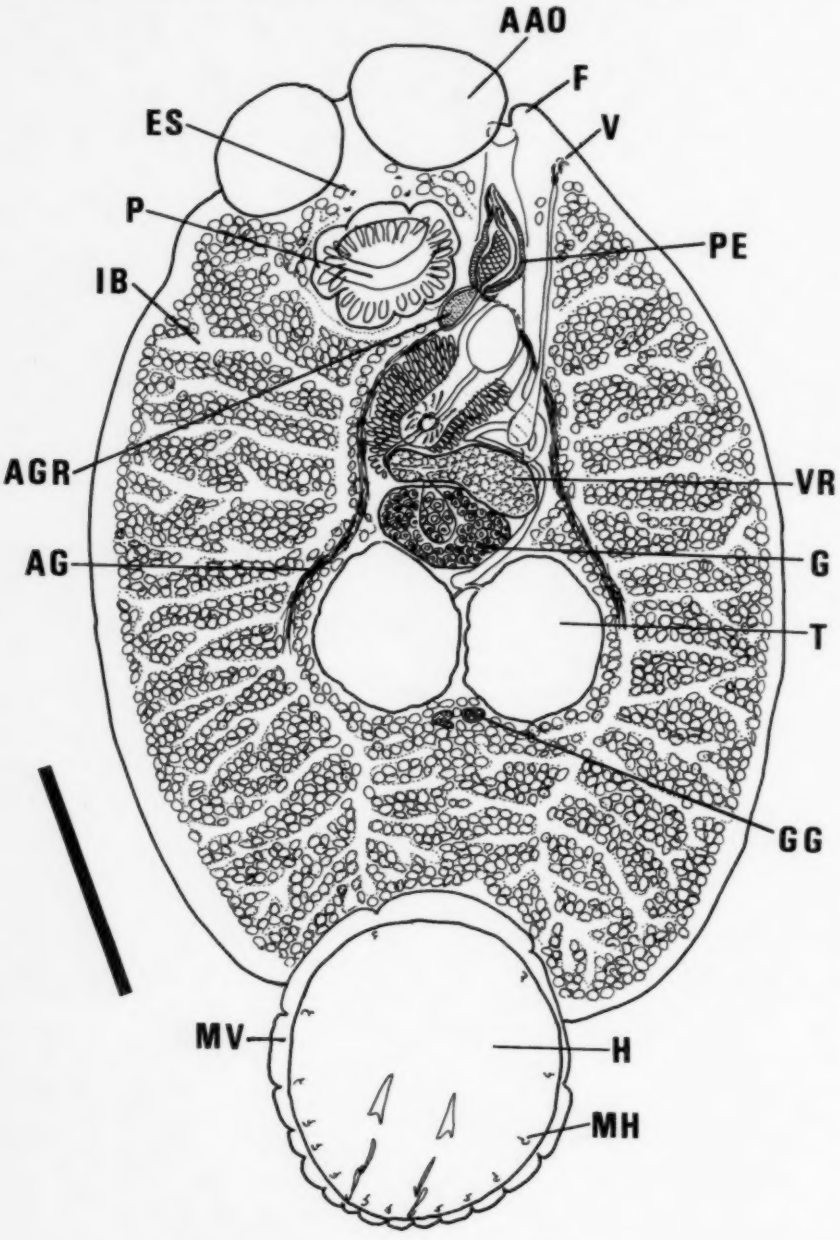
Type locality: Inland Sea of Japan.

Site of infection: Fins, eyes, and body surface.

Material examined

Redescription based on 28 mounted specimens collected from the fins and body surface of Japanese flounder, *Paralichthys olivaceus*, from Shimane Prefecture in October, 1989 and from Oita Prefecture in July, 1992. For comparative

Fig. 1. *Benedenia epinepheli* (Yamaguti, 1937), ex. *Paralichthys olivaceus*, Oita Prefecture, Japan, July 1992. Entire animal in ventral view. AAO, anterior attachment organ; AG, accessory glands; AGR, accessory gland reservoir; ES, eyespot; F, flap; G, germarium; GG, glands of Goto; H, haptor; IB, intestinal branch; MH, marginal hook; MV, marginal valve; P, pharynx; PE, penis; T, testis; V, vagina; VR, vitelline reservoir. Scale bar = 0.5 mm.



purposes, the following material was examined: *Epibdella epinepheli* Yamaguti, 1937, holotype and one paratype ex. *Epinephelus akaara*, Meguro Parasitological Museum (MPM) Coll. No. 22771; *Benedeniella congeri* Yamaguti, 1958, holotype ex. *Conger myriaster*, MPM Coll. No. 22356; *Benedenia* sp. of Tsutsumi and Ito, 1965 ex. unspecified aquarium fishes, personal collection of T. Tsutsumi, Shimonoseki Aquarium, Yamaguchi Prefecture, Japan (12 specimens); and *Neobenedeniella congeri* (Yamaguti, 1958), material of Kamegai et al. (1985) ex. *Alutera*

Table 2. Comparative morphometrics of *Benedenia epinepheli* (Yamaguti, 1937) (all measurements in mm).

Parameters[b]	*Epibdella epinepheli* Yamaguti, 1937 (holotype and 1 paratype) Host: *Epinephelus akaara* (n = 2)	*Benedeniella congeri* Yamaguti, 1958 (holotype) *Conger myriaster* (n = 1)	*Benedenia* sp. of Tsutsumi & Ito, 1965 Not specified (n = 12)	*Neobenedeniella congeri* of Kamegai, Yasunaga, & Hirakawa, 1985 *Aluterus monoceros* (n = 7)
1*a*	1.85–2.02	1.35	1.70–3.00	1.17–2.20
1*b*	0.80–0.85	0.50	0.72–1.25	0.57–1.25
2*a*	0.41–0.42	0.38	0.50–0.70	0.34–0.57
2*b*	0.41–0.42	nm[c]	0.51–0.68	0.27–0.52
3*a*	nm	0.12–0.13	0.16–0.51	0.11–0.24
3*b*	nm	0.10–0.12	0.17–0.49	0.09–0.23
4*a*	0.070–0.092	0.060–0.070	0.080–0.110	0.054–0.096
4*b*	0.080–0.100	0.070	0.086–0.130	0.056–0.092
4*c*	0.050–0.062	0.056–0.070	0.066–0.096	0.022–0.076
5*a*	0.14	0.12	0.12–0.34	0.08–0.18
5*b*	0.18–0.19	0.12	0.13–0.35	0.10–0.27
6*a*	0.19	0.09	0.08–0.23	0.09–0.19
6*b*	0.25	0.13	0.15–0.27	0.16–0.26
7*a*	0.33–0.35	0.30	0.29–0.49	0.29–0.38
7*b*	0.23–0.28	0.17–0.18	0.19–0.34	nm
8*a*	0.32–0.35	0.29	0.23–0.40	0.23–0.43
8*b*	0.06–0.08	0.06	0.05–0.10	0.05–0.09
Source	MPM coll. No. 22771	MPM coll. No. 22356	MPM coll. No. 19658	MPM coll. Nos. 1041–1048

[a]Parameters: 1*a* and 1*b*, body length and width; 2*a* and 2*b*, haptor length and width; 3*a* and 3*b*, anterior attachment disc length and width; 4*a*, accessory sclerite length; 4*b*, anterior hamulus length; 4*c*, posterior hamulus length; 5*a* and 5*b*, pharynx length and width; 6*a* and 6*b*, ovary length and width; 7*a* and 7*b*, testes length and width; 8*a* and 8*b*, penis length and width.
[b]No. of specimens measured.
[c]nm, not measured.

(=*Aluterus*) *monoceros*, MPM Coll. Nos. 1041–1048 (7 specimens). Illustrations were based on specimens of the following: *B. epinepheli* ex. *P. olivaceus* from Oita and Shimane Prefectures; *Oplegnathus punctatus* and *Sebastes marmoratus* from Hyogo Prefecture; *Benedeniella congeri*, holotype; *Neobenedeniella congeri*, material of Kamegai et al. (1985); and *Benedenia* sp. of Tsutsumi and Ito (1965).

Deposition of specimens

Voucher specimens of *B. epinepheli* from Japanese marine fishes are deposited in the collections of the United States National Parasite Collection, USDA, Beltsville, Md., as USNM No. 84307 and the Meguro Parasitological Museum, Tokyo, Japan, as MPM Coll. Nos. 19646–19657; while those identified as *Benedenia* sp. by Tsutsumi and Ito (1965) are deposited in the MPM as MPM Coll. No. 19658.

Redescription

Total body length including haptor 1.5–3.1 (2.1 ± 0.4); maximum width 0.78–1.58 (1.06 ± 0.20). Anterior attachment organs disc-like, 0.16–0.32 (0.23 ± 0.03) long, 0.19–0.37 (0.27 ± 0.03) wide. Flap-like structure prominent, slightly left of common genital aperture (Fig. 1).

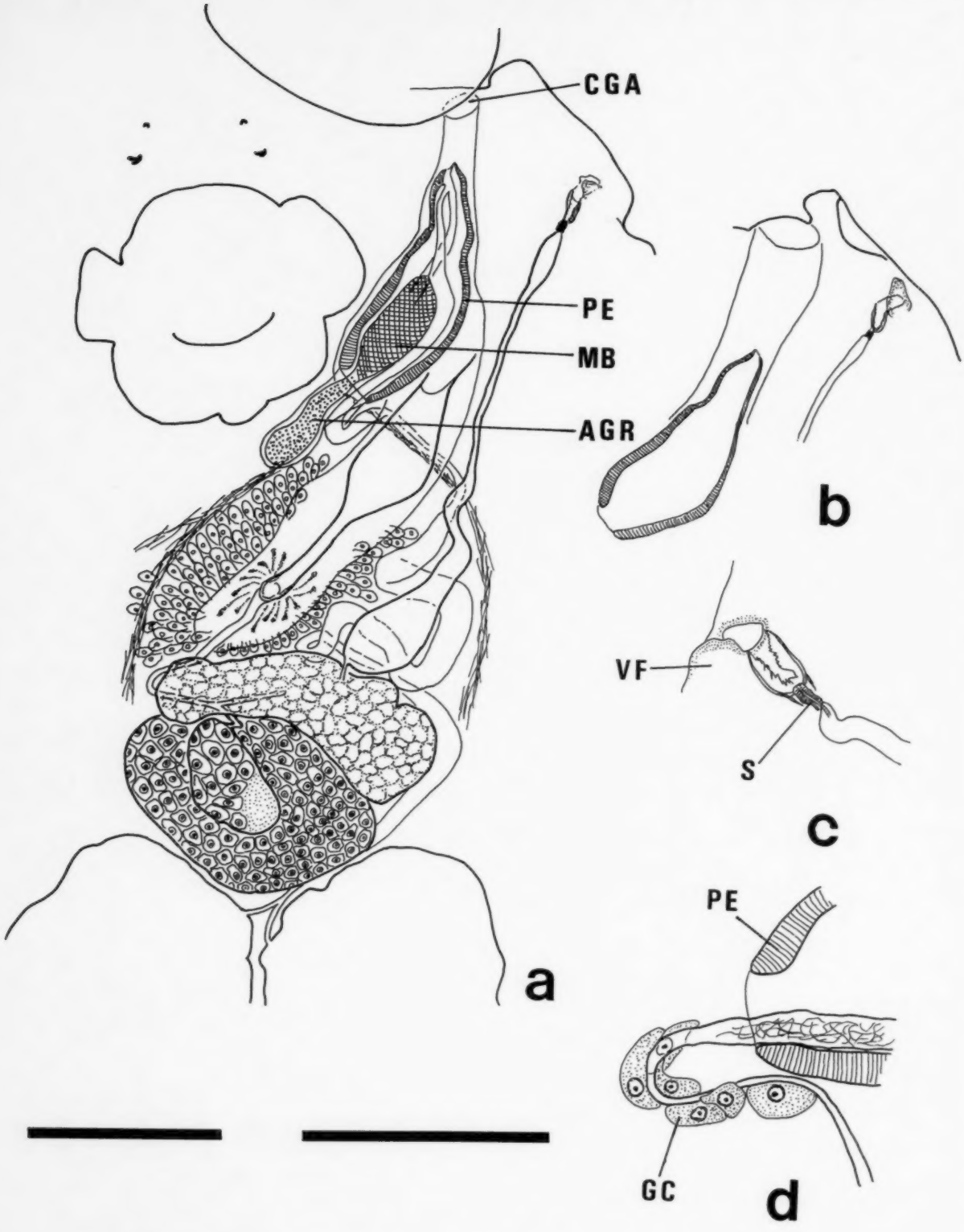

CGA
PE
MB
AGR
a
b
VF
S
c
PE
GC
d

Fig. 2. Detailed reproductive system of *B. epinepheli* (Yamaguti, 1937), ex. *Paralichthys olivaceus*. (*a*) Whole reproductive system, ventral view (Oita Pref., July 1992). (*b*) Details of flaps associated with common genital aperture and vaginal pore, ventral view (Oita Pref., July 1992). (*c*) Distal end of vagina showing posterior flap and sphincter, dorsal view (Shimane Pref., October 1989). (*d*) Gland cells associated with vas deferens, ventral view (Oita Pref., July 1992). AGR, accessory gland reservoir; CGA, common genital aperture; GC, gland cells; MB, muscular bulb; PE, penis; S, sphincter; VF, vaginal flap. Left scale bar = 0.2 mm and applies to Figs. 2*a* and 2*b*; right scale bar = 0.1 mm and applies to Figs. 2*c* and 2*d*.

Haptor elliptical, 0.43–0.70 (0.54 ± 0.08) long, 0.40–0.75 (0.52 ± 0.09) wide. Marginal valve scalloped, lobes wider and broader anteriorly than posteriorly (Fig. 1). Median sclerites (Fig. 3*a*) consist of pair of accessory sclerites, anterior hamuli, and posterior hamuli. Accessory sclerites and anterior hamuli nearly equal in length. Accessory sclerites 0.040–0.086 (0.075 ± 0.010) long, slightly curved inward, tapering anteriorly to blunt tip, posterior end notched with blunt tips. Anterior hamuli 0.062–0.100 (0.080 ± 0.010) long, relatively stout, with blunt anterior end and strongly recurved posterior hook. Posterior hamuli 0.044–0.070 (0.057 ± 0.006) long, with finely curved posterior hook, widening anteriorly into spatulate anterior end. Fourteen marginal hooks arranged radially. Single indentation of marginal valve corresponds to each marginal hook and posterior hamulus (Fig. 1). Two pairs of eyespots behind anterior attachment discs. Lobulate pharynx immediately behind eyespots, 0.13–0.34 (0.22 ± 0.05) long by 0.17–0.38 (0.27 ± 0.04) wide. Main branches of intestine extend medially and posteriorly from about level of eyespots to end of body proper, terminating blindly with numerous secondary and tertiary branches. Glands of Goto small, unequal in size and irregularly shaped, with many nuclei, post-testicular. Testes juxtaposed, sometimes contiguous, 0.10–0.41 (0.32 ± 0.05) long, 0.18–0.34 (0.25 ± 0.03) wide. Narrow vas efferens emerges from each testis to join vas deferens behind ovary. Vas deferens ascends sinistral to ovary and vitelline reservoir with several undulations; six gland cells of unknown function attach just before entry to penis (Fig. 2*d*); vas deferens inside penis joins with duct from accessory gland reservoir near tip of penis. Penis sinistral to pharynx, 0.26–0.45 (0.35 ± 0.04) long, 0.065–0.100 (0.091 ± 0.010) wide, with muscular wall, bulbous in posterior two-thirds, narrowing anteriorly to pointed tip. Muscular bulb of accessory gland occupies posterior one-third of penis; accessory gland reservoir thin-walled, receives accessory gland from both ends of reservoir, one bundle entering at proximal end of wall, the other near entry point of vas deferens. Germarium transversely elongated, 0.09–0.20 (0.15 ± 0.03) long, 0.14–0.37 (0.23 ± 0.04 wide, anterior to testes, with internal fertilization chamber. Germiduct emerges from fertilization chamber to connect with vitelline reservoir by short duct, passes dextral to vitelline reservoir before expanding to form triangular ootype. Mehlis' glands empty at junction of germiduct and ootype. Uterus short, joins penis near common genital aperture. Common genital aperture opens ventrally on anterolateral margin of body, just anterior to eyes on left side of body. Vagina communicates with vitelline reservoir via narrow duct forming seminal receptacle proximally; vaginal duct long, narrow, at distal end slightly sclerotized, funnel shaped; vaginal pore provided with posterior sphincter (Figs. 2*a*–2*c*), opening dorsally near left margin of body posterior to common genital aperture. Small flap present posterior to vaginal pore (Figs. 2*a*–2*c*). Vitellaria coexist with intestine from level of eye to end of body proper. Egg tetrahedral, 0.10–0.12 (0.11 ± 0.01) long, 0.09–0.11 (0.09 ± 0.01) wide, with long filament.

Comments

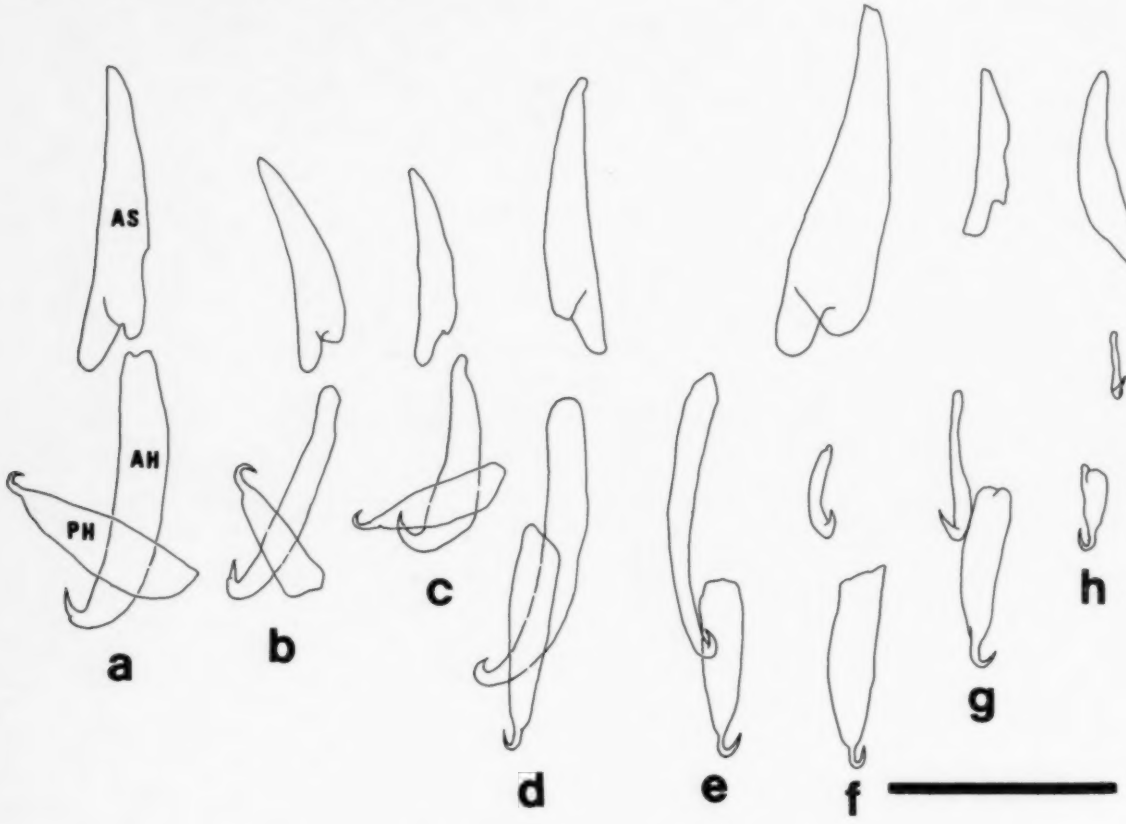
Benedenia epinepheli (Yamaguti, 1937) was first described as *Epibdella epinepheli* from the gills of wild redspotted grouper, *Epinephelus akaara* (Yamaguti 1937). Subsequently, it was transferred to the genus *Benedenia* by Meserve (1938). *Benedeniella congeri* Yamaguti, 1958 was described by Yamaguti (1958) based on a single specimen collected from the whitespotted conger, *Conger myriaster*. However, as the genus *Benedeniella* was preoccupied, a new genus, *Neobenedeniella*, was erected by Yamaguti (1963) to accomodate *B. congeri*. This species was subsequently reported by Kamegai et al. (1985) on the eyes and fins of unicorn filefish, *Alutera* (=*Aluterus*) *monoceros*, from Nagasaki Prefecture. Byrnes (1986) considered the genus *Neobenedeniella* a junior synonym of *Benedenia*, indicating that the differentiating characters, a prostatic reservoir (= accessory gland) enclosed in the cirrus pouch and a posteriorly bifid anterior hamulus, are both common in species of *Benedenia*.

Examination of the type specimens of *Benedenia epinepheli* Yamaguti, 1937 and *Benedeniella congeri* Yamaguti, 1958, and specimens identified as *Neobenedeniella congeri* by Kamegai et al. (1985) and as *Benedenia* sp. by Tsutsumi and Ito (1965) showed similarities in the reproductive organs and median sclerites and comparable morphometrics (Table 2). All showed the characteristic flap slightly left of the common genital aperture. Although this flap is not very prominent in the holotype of *B. congeri*, this may be because it has been slightly folded or has contracted during fixation. These findings show that *Benedeniella congeri* Yamaguti, 1958, *Neobenedeniella congeri* (Yamaguti, 1958), and *Benedenia* sp. of Tsutsumi and Ito, 1965 are junior synonyms of *Benedenia epinepheli* (Yamaguti, 1937).

Morphological variations observed in the haptoral sclerites of *B. epinepheli* are shown in Figs. 3*e*–3*h*. These include absence of the accessory sclerite (Fig. 3*e*); and poor development of the anterior hamulus (Fig. 3*f*), accessory sclerite and posterior hamulus (Fig. 3*g*), and anterior and posterior hamuli (Fig. 3*h*).

Six species of cultured marine fishes and seven species of marine aquarium fishes of Japan are new hosts for this parasite (see Table 1). *Benedenia epinepheli* was recovered on different species of aquarium fishes in Yomiuri Land Marine Aquarium, Tokyo Metropolis, in Suma Aqualife Park, Hyogo Prefecture, and in Shimonoseki Aquarium in Yamaguchi Prefecture; and on cultured marine fishes in eight

Fig. 3. Morphology of half pair of median sclerites. (*a–d*) Normal shapes of median sclerites. (*e–h*) Variations. (*a*) *B. epinepheli* (Yamaguti, 1937), ex. *Oplegnathus punctatus*, Hyogo Pref., December 1989. (*b*) *B. epinepheli* paratype, ex. *Epinephelus akaara*. (*c*) *Benedeniella congeri* Yamaguti, 1958 holotype, ex. *Conger myriaster*. (*d*) *Benedenia* sp. of Tsutsumi and Ito, 1965, ex. unspecified aquarium fish. (*e*) Loss of accessory sclerite, ex. *Paralichthys olivaceus*, Oita Pref., July 1992. (*f*) Poorly developed anterior hamulus, ex. *Sebasticus marmoratus*, Hyogo Pref., October 1989. (*g*) Poorly developed accessory sclerite and anterior hamulus, ex. *P. olivaceus*, Oita Pref., July 1992. (*h*) *Neobenedeniella congeri* (Yamaguti, 1958), material of Kamegai et al. (1985), unflattened accessory sclerite and poorly developed anterior and posterior hamuli, ex. *Aluterus monoceros*, Nagasaki Pref., January 1984. AH, anterior hamulus; AS, accessory sclerite; PH, posterior hamulus. Scale bar = 0.1 mm.



prefectures from three major islands of Japan: in Honshu (Fukui, Wakayama, Shimane, and Yamaguchi), in Shikoku (Ehime), and in Kyushu (Oita and Kagoshima) (Fig. 4).

In January 1993, during investigation of a disease outbreak in Japanese flounder at Ehime Prefecture, a maximum intensity of 3000 parasites was found on a fish weighing 670 g. The parasites, quite difficult to detect but readily visible after freshwater bath treatment, were found on and around the eyes and the mouth (Fig. 5*b*) and on the fins, where they caused haemorrhagic and abrasive lesions. At least two cases of mortality were associated with this parasite: first, in October 1989, among cultured flounder in Shimane and secondly, in September 1992, on 2-year-old black rockfish (*Sebastes schlegeli*) in Yamaguchi Prefecture, where 700 mortalities were recorded and intensities were 900–1500 worms/fish.

Discussion

This description of *B. epinepheli* presents features previously undescribed in the original (Yamaguti 1937) and subsequent (Tsutsumi and Ito 1965) descriptions of this parasite. These features include the flaps associated with the common genital opening and vaginal pore, the scalloped marginal valve with a single indentation corresponding to each marginal hook and posterior hamulus, and the proportion and shapes of the median sclerites.

The presence of flaps (Hussey 1986) or lobes (Whittington and Kearn 1993) has been reported in other benedeniine species. For instance, in *Tareenia acanthopagri* Hussey, 1986, such a flap overlies the genital pore, and it was suggested that it may be concerned with holding a spermatophore before transfer or with accumulation of and

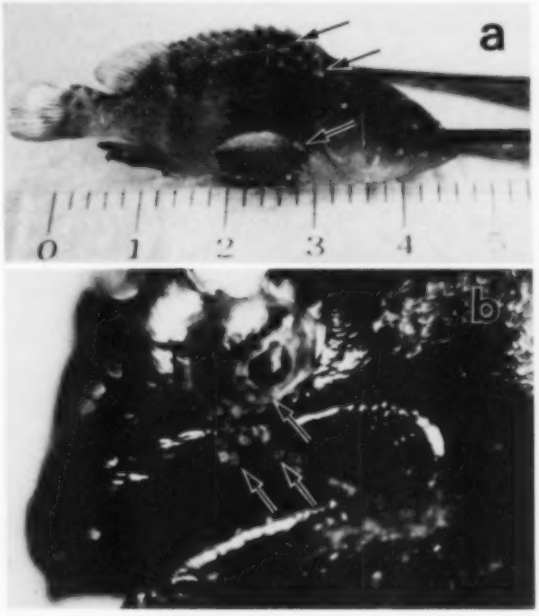
Fig. 4. Map of southwestern Japan showing geographical distribution of *B. epinepheli* collected from both aquarium (▲) and cultured (●) marine fishes. Letters indicate the following: Honshu Island: (*a*) Tokyo, (*b*) Fukui, (*c*) Wakayama, (*d*) Hyogo, (*e*) Shimane, (*f*) Yamaguchi. Shikoku Island: (*g*) Ehime. Kyushu Island: (*h*) Oita, (*i*) Kagoshima.

protection of discharged eggs (Hussey 1986). In *Benedenia lutjani* Whittington and Kearn, 1993, a pair of lobes associated with the common genital aperture and a single lobe associated with the vaginal opening were described; it was suggested that these lobes may be sensory, serving to align the genital regions of co-copulants prior to and during mating (Whittington and Kearn 1993). The flaps in *T. acanthopagri*, B. *lutjani*, and *B. epinepheli* differ mainly in their relation to the position of the genital organs with which such lobes or flaps are associated. The association of these flaps with the reproductive organs of the parasite indicates their possible reproductive function, perhaps acting as a guide during mating or as protection for these organs.

The scalloped marginal valve present on the haptoral margin of *B. epinepheli* has been observed in at least three other benedeniine species, namely, *B. anticavaginata* Byrnes, 1986; *B. lutjani*; and *T. acanthopagri*. Whittington and Kearn (1993) indicated that the arrangement of scallops and marginal hooks is similar for the above species. This arrangement differs markedly from that found in *B. epinepheli*, where the scallops are wider and broader anteriorly than posteriorly, with a single indentation corresponding to each marginal hook and posterior hamulus. This scalloped marginal valve may have some taxonomic importance, as Whittington and Kearn (1993) suggested. However, such features of the marginal valve are difficult to observe except on well-flattened or living specimens.

This is the first time that *Benedenia epinepheli* has been reported from cultured marine fishes of Japan, the original record of this parasite in 1937 being from wild Japanese fish. Since 1989, the parasite has been recovered on the fins, eyes, and body surface of six species of cultured Japanese marine fishes (Figs. 5*a* and 5*b*), while it continues to infect more species in marine aquaria. A total of 25 fish species serve as hosts for this parasite (Table 1), the most susceptible being tetraodontiform fishes, with 11 species from five families. The finding of *B. epinepheli*

Fig. 5. Fish infected with *B. epinepheli*. (*a*) *Epinephelus akaara* infected on fins and body surface (arrows), Fukui Pref., December 1990, formalin-fixed specimen. Scale in centimetres. (*b*) *Paralichthys olivaceus* heavily infected on and around eye and mouth region (arrows), Ehime Pref., January 1993. Scale bar = 1 cm.



on Japanese flounder is the first report of a species of *Benedenia* from fishes of the order Pleuronectiformes. The very low host specificity of *B. epinepheli* is quite exceptional for monogeneans, which are generally very host specific (Shulman 1958). Among members of the genus *Benedenia*, *B. epinepheli* and *B. hawaiiensis* Yamaguti, 1968 seem to be the only two species with a very wide host range. Yamaguti (1968) listed 24 hosts for *B. hawaiiensis*. Two species of the genus *Neobenedenia* Yamaguti, 1963, namely, *N. melleni* (MacCallum, 1927) and *N. girellae* (Hargis, 1955), infect many species of marine cultured and aquarium fishes (see, for example, Jahn and Kuhn 1932; Nigrelli 1937, 1947; Ogawa et al. 1995).

The variations in the morphology of individual parts of the haptoral complex observed for this parasite have been noted previously in *Neobenedenia girellae* (Ogawa et al. 1995). It is not clear whether these variations are normal, temperature related, or resulting from differences in the parasite's response to different hosts.

The ability of *B. epinepheli* to cause death of cultured fishes during heavy infection, and its low host specificity, ubiquitous distribution in western Japan, and the high susceptibility of marine aquarium fishes are indicative of the parasite's great potential to become a disease problem.

Acknowledgements

We thank the following for help in collection of parasite specimens: staff of the Japanese Prefectural Fisheries

Experimental Stations in Fukui, Wakayama, Shimane, Yamaguchi, Ehime, Oita, and Kagoshima; Mr. H. Sanagawa, Suma Aqualife Park, Kobe, Hyogo Prefecture; and Mr. M. Iwata, Yomiuri Land Marine Aquarium, Tokyo. Thanks also go to Mr. T. Tsutsumi, Shimonoseki Aquarium, Yamaguchi Prefecture, for kindly providing specimens of *Benedenia* sp. from his private collection, and to Dr. S. Kamegai, Director, Meguro Parasitological Museum, Tokyo, for the loan of type specimens.

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Steringophorus Odhner, 1905 (Digenea: Fellodistomidae) in deep-sea fishes from the northeastern Atlantic, with the description of *Steringophorus margolisi* n.sp.

Rodney A. Bray

Abstract: ***Steringophorus margolisi*** n.sp., described from the ophidiid *Spectrunculus grandis*, is distinguished by its elongate vitelline fields and long caeca. ***Steringophorus*** (=*Occultacetabulum*) ***dorsolineatum*** (Reimer, 1985) n.comb. is described from the chlorophthalmid *Bathypterois dubius*. It is suggested that the genus *Occultacetabulum* is synonymous with *Steringophorus*, and evidence is presented to show that the withdrawal of the ventral sucker into the body is a temporary condition. *Steringophorus thulini* Bray and Gibson, 1980 is partly redescribed from the macrourids *Coryphaenoides* (*Chalinura*) *mediterraneus*, *C.* (*Chal.*) *brevibarbis*, *C.* (*Chal.*) *leptolepis*, *C.* (*Chal.*) *profundicolus*, and *Trachyrincus murrayi*. *Steringophorus blackeri* Bray, 1973 is reported from the alepocephalid *Xenodermichthys copei*.

Résumé : ***Steringophorus margolisi*** n.sp., reconnaissable à ses champs vitellins étendus et à ses caeca allongés, est décrit d'après des individus trouvés sur l'Ophidiidé *Spectrunculus grandis* et ***Steringophorus*** (=*Occultacetabulum*) ***dorsolineatum*** (Reimer, 1985) n.comb., d'après des spécimens prélevés sur *Bathypterois dubius*. L'auteur propose de traiter le genre *Occultacetabulum* comme synonyme de *Steringophorus*. Il démontre également que le retrait de la ventouse ventrale dans le corps est un état provisoire. *Steringophorus thulini* Bray et Gibson, 1980 fait l'objet d'une redescription partielle d'après des individus trouvés sur les Macrouridés *Coryphaenoides* (*Chalinura*) *mediterraneus*, *C.* (*Chal.*) *brevibarbis*, *C.* (*Chal.*) *leptolepis*, *C.* (*Chal.*) *profundicolus*, et *Trachyrincus murrayi*. L'association de *Steringophorus blackeri* Bray, 1973 avec l'Alépocéphalidé *Xenodermichthys copei* est signalée.

[Traduit par la Rédaction]

The deep sea holds a distinctive fauna of fishes, many of them belonging to the family Gadiformes, and it is becoming apparent that this fauna holds a distinctive suite of digenean parasites. The major groups represented are Lepocreadiidae Odhner, 1905 of the subfamily Lepidapedinae Yamaguti, 1958 (see Bray and Gibson 1995) and Fellodistomidae Nicoll, 1909 of the subfamily Fellodistominae Nicoll, 1909. In this paper I report some new observations on members of the genus *Steringophorus* Odhner, 1905, the largest genus of the latter subfamily, from the northeastern Atlantic, mainly from gadiforms, but also from myctophiforms and salmoniforms. This genus includes the following species: *S. furciger* (Olsson, 1868); *S. blackeri* Bray, 1973; *S. brevis* (Ching, 1960); *S. congeri* Shen, 1987; *S. foliatus* (Yamaguti, 1970); *Steringophorus* n.sp. of Bray and Campbell, 1995; *S. magnus* Manter, 1934; *S. melanostigma* (Noble and Orias, 1975); *S. pritchardae* (Campbell, 1975); *S. profundus* Manter, 1934 and *S. thulini* Bray and Gibson, 1980; Bray et al. (1994) have redefined the genus using rRNA sequences and transferred two other species to the genus *Fellodistomum* Stafford, 1904.

The only well-known species is *S. furciger*, which has a large number of records, mainly from pleuronectiforms in relatively shallow (shelf) waters (Bray and Campbell 1995). Of the other species seven have only been reported once, and none are well known. Several are found in deep water; namely, *S. blackeri*, *Steringophorus* n.sp. of Bray and Campbell, 1995, *S. magnus*, *S. melanostigma*, *S. pritchardae*, *S. profundus*, and *S. thulini*.

Received December 21, 1993. Accepted February 11, 1994. J12491

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Materials and methods

The material was collected on board the NERC vessels RRS *Discovery* and RRS *Challenger* by myself, Mary

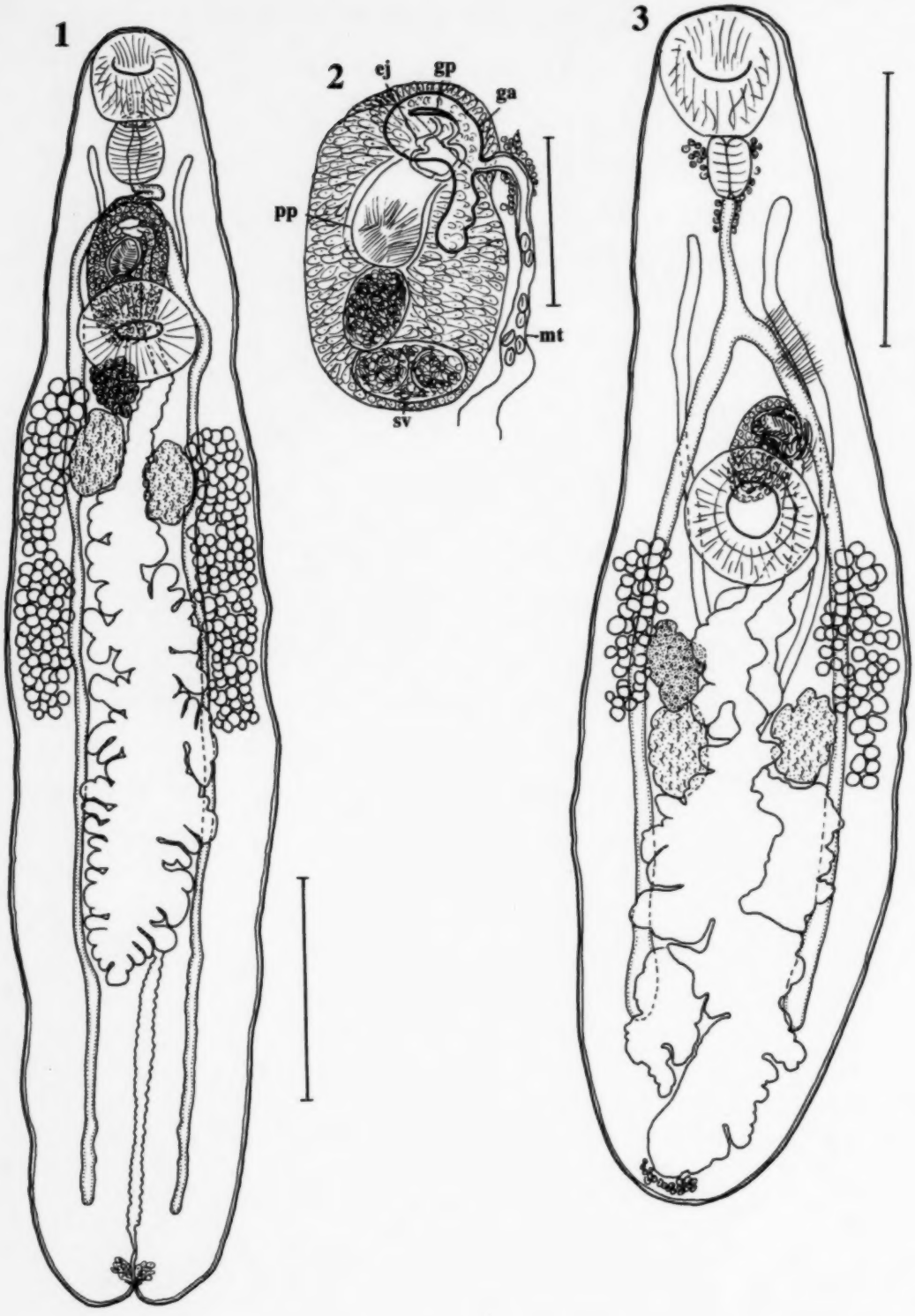
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Fig. 1. *Steringophorus margolisi* n.sp., ventral view of holotype, uterus in outline. **Fig. 2.** *Steringophorus margolisi* n.sp., terminal genitalia. **Fig. 3.** *Steringophorus dorsolineatum*, ventral view of specimen from *Bathypterois dubius*, uterus in outline. *ej*, ejaculatory duct; *ga*, genital atrium; *gp*, genital pore; *mt*, metraterm; *pp*, pars prostatica; *sv*, seminal vesicle. Scale bars: Fig. 1, 1000 μm; Fig. 2, 300 μm; Fig. 3, 500 μm.

Spencer Jones, and Sue Mitchell. Live worms were fixed in Berland's fluid (Gibson 1979) and preserved in 80% ethanol. Whole mounts were stained in Mayer's paracarmine and mounted in Canada balsam. The gadiforms nomenclature used in this paper is according to Cohen et al. (1990).

Measurements are given in micrometres as the range, with the mean in parentheses.

Results

Family Fellodistomidae Nicoll, 1909
Fellodistominae Nicoll, 1909: 458

Subfamily Fellodistominae Nicoll, 1909
Fellodistominae Nicoll, 1909: 458
Steringophoridae Odhner, 1911: 98
Lissolomatinae Skrjabin and Koval, 1957: 365
Markevitschiellinae Skrjabin and Koval, 1957: 378
Occultacetabulinae Reimer, 1985: 108

Genus *Steringophorus* Odhner, 1905
Steringophorus Odhner, 1905: 309 (type species *S. furciger* (Olsson, 1868) by original designation)
Leioderma Stafford, 1904: 486 *nec* Willemoes-Suhm, 1873 (type species *L. furcigerum* (Olsson, 1868) by monotypy)
Callodistomoides Yamaguti, 1970: 43 (type species *C. foliatus* Yamaguti, 1970 by original designation)
Abyssotrema Campbell, 1975: 661 (type species *A. pritchardae* Campbell, 1975 by original designation)
Occultacetabulum Reimer, 1985: 108 (type species *O. dorsolineatum* Reimer, 1985 by original designation)

***Steringophorus margolisi* n.sp.** Figs. 1–2

Material studied

ex *Spectrunculus grandis* (Günther), Gadiformes: Ophidiidae. Intestine. Rockall Trough (56°44′N, 09°45′W; depth 1745–1760 m; 19.11.1991. Collector R.A. Bray). BM(NH) holotype 1992.3.24.10, paratypes 1992.3.24.11–14.

Description (based on four mature and one immature whole-mounted specimens and one serially sectioned specimen)

Body elongate, linguiform; 4659–5840 × 1072–1296 (5053 × 1220); width 21–28 (24)% of length (Fig. 1). Tegument unarmed. Oral sucker subglobular, subterminal; 393–412 × 392–419 (401 × 407). Ventral sucker transversely oval; 441–489 × 489–535 (464 × 518). Sucker ratio (1:1.23–1.33 (1.27). Forebody 1008–1128 (1065) long, 9–23 (21)% of body length. Prepharynx short, within posterior concavity of oral sucker. Pharynx oval, large; 316–344 × 251–270 (327 × 261). Oesophagus distinct, recurved; surrounded by gland-cells anteriorly; distance from pharynx to intestinal bifurcation 109–142 (134). Intestinal bifurcation in posterior forebody, dorsal to anterior part of cirrus-sac; 232–287 (262) from ventral sucker. Caeca long, reaching posteriorly to uterus, terminate blindly 395–481 (427) from posterior extremity.

Testes 2; margins irregular to smooth, not deeply lobed; oblique to almost symmetrical; in anterior hindbody; anterior 316–406 × 213–322 (358 × 278), posterior 303–406 × 225–328 (370 × 256). Posttesticular region of body 2594–3552 (2899) long; 56–61 (57)% of body length. Cirrus-sac large, 534–612 × 361–386 (578 × 370); oval, overlaps to posterior half of ventral sucker (Fig. 2). Seminal receptacle bipartite, moieties oval; in proximal cirrus-sac. Pars prostatica vesicular; lined with long filaments. Ejaculatory duct muscular, complexly folded. Genital atrium large, muscular, complexly folded; surrounded by gland-cells. Genital pore ventro-median, at level of intestinal bifurcation.

Ovary multilobate; dextrally submedian, overlapping posterior edge of ventral sucker; 219–296 × 161–238 (261 × 191). Mehlis' gland postero-dorsal to ovary, dorsal to anterior testis. Laurer's canal opens dorsally to anterior testis. Uterine seminal receptacle formed in initial coils of uterus. Uterus long, convoluted; intercaecal, extends posteriorly from ovary forming lateral coils to 552–1472 (933) (12–25 (18)% of body length) from posterior extremity, then passes forward forming lateral coils, passes between testes, dorsal to sinistral side of ventral sucker. Metraterm muscular; surrounded by gland-cells. Eggs numerous; initially light yellow but darken to deep brown in ascending uterine coils, with pattern of fine tubercles on shell; operculate; 42–47 × 19–31 (44 × 27). Vitellarium follicular, large fields 1184–1648 (1366) long, with anterior extent close to posterior margin (129 anterior to 167 posterior (34 anterior)) of ventral sucker; posterior limit well posterior to testes.

Excretory pore terminal. Vesicle Y shaped; initially narrow and surrounded by gland-cells; widens, with wrinkled wall, passes forward medially, reaches almost to testes bifurcation obscured by eggs and also not apparent in immature specimen; arms reach to about level of mid-pharynx.

Discussion

This species exhibits two strikingly unusual features for members of the genus *Steringophorus*. These are the elongate vitelline fields, the bulk of which lies posterior to the testes, and the relatively long caeca, which extend posteriorly to the uterus. One other species, *S. pritchardae* (Campbell, 1975), has similarly elongate vitelline fields, but the tandem testes lie close to their posterior limit (Campbell 1975; Bray and Gibson 1980). This species also differs in that the caeca do not extend posteriorly to the uterus.

The only other species in the genus to be reported from ophidiid hosts is *Steringophorus* n.sp. of Bray and Campbell, 1995, from *Porogadus milesi*, in the northwestern Atlantic (Bray and Campbell 1995). Apart from the features mentioned above, this species differs from *S. margolisi* n.sp. in its more squat body, its smaller pharynx (174–187 × 155–187), its uterus that reaches close to the posterior extremity and its smaller eggs (35–41 × 19–22).

The new species is named for Dr. Leo Margolis, the laureate of this volume, in recognition of his major contributions to marine parasitology.

***Steringophorus dorsolineatum* (Reimer, 1985) n.comb.**
Fig. 3

Occultacetabulum dorsolineatum Reimer, 1985: 107, Figs. 1–2.

Material studied

ex *Bathypterois dubius* Vaillant, Myctophiformes: Chlorophthalmidae (incl. Bathypteroidae). Intestine. Goban Spur (49°39′N, 12°08′W, depth 1315–1295 m, 20.8.1989. Collector R.A. Bray), BM(NH) 1990.6.14.9-10; Rockall Trough (56°43′N, 09°26′W, 1360–1520 m, 21.2.1991. Collector M.E. Spencer Jones), BM(NH) 1992.7.28.5–6.

ex *Bathypterois ater* Gilchrist, Myctophiformes: Chlorophthalmidae. Intestine. Mozambique. Two specimens lent by Dr. L. Reimer.

Description (based on seven whole-mounted specimens and one sectioned specimen from *B. dubius*)

Body elongate-pyriform; 1450–2304 × 406–599 (1996 × 533); width 22–33 (27)% of length. Tegument unarmed; patch of diagonal muscular ridges on ventral surface on sinistral forebody, just anterior to genital pore (Fig. 3); strong musculature also present just beneath body wall surrounding genital atrium and ventral sucker. Oral sucker oval, subterminal; 185–245 × 170–238 (226 × 214). Ventral sucker usually rounded; 206–269 × 180–245 (242 × 220); aperture usually on ventral surface; in one worm ventral sucker withdrawn into body and distorted to irregularly oval, leaving narrow slit-like aperture on ventral surface just postero-dextral to genital pore. Sucker ratio 1:0.90–1.18 (1.03). Forebody 460–832 (700) long, 31–37 (35%) of body length. Prepharynx not seen. Pharynx oval; 82–116 × 70–104. Oesophagus long, 75–238 (165); surrounded by gland cells anteriorly. Intestinal bifurcation in posterior forebody; 135–316 (216) from ventral sucker. Caeca reach into posterior half of posttesticular region, 241–391 (312) from posterior extremity.

Testes 2; subsymmetrical, in anterior third of hindbody; weakly lobate; 103–206 × 70–129 (167 × 100). Posttesticular region of body 505–904 (701) long; 32–39 (35)% of body length. Cirrus-sac elongate oval, small; 142–200 × 71–129 (173 × 109); overlaps anterior half of ventral sucker; proximal part lies horizontally in whole mount; distal part recurved towards ventral surface. Seminal vesicle saccular, may appear bipartite, tripartite, convoluted, or rectilinear; fills much of proximal cirrus-sac. Pars prostatica vesicular, oval; lined with long filaments. Seminal vesicle and pars prostatica surrounded by gland-cells. Ejaculatory duct complexly folded, with thick muscular walls continuous with wall of genital atrium. Genital atrium large, muscular. Genital pore antero-sinistral to ventral sucker or, when ventral sucker is withdrawn, antero-sinistral to slit-like opening of cavity produced by withdrawn ventral sucker.

Ovary weakly multilobate, immediately anterior to right testis; 58–112 (89) from ventral sucker; 116–174 × 52–129 (142 × 97). Laurer's canal not seen (sections damaged at critical point). Uterus passes anteriorly from ovary, initially forming uterine seminal receptacle; just overlaps ventral sucker before turning posteriorly, running between testes and forming mainly intercaecal lateral coils in posttesticular region, reaches close to posterior extremity, then passes forward forming more mainly intercaecal lateral coils, extends dorsally to sinistral part of ventral sucker. Metraterm short, muscular; enters genital atrium sinistrally. Eggs numerous, operculate; 45–54 × 22–29 (48 × 27), shell colourless initially, darkening through bright yellow to dark brown as eggs pass along uterus. Vitellarium follicular; small lateral fields reach from posterior half of ventral sucker to level of testes or just beyond.

Excretory pore terminal. Vesicle Y shaped; narrow initially, surrounded by gland-cells, then forms small fusiform chamber before narrowing again and passing forward medially to just posterior to testes, where it divides giving off arms which pass antero-laterally, terminating laterally at level of oesophagus.

Records: 1. Reimer (1985); 2. Present study.

Descriptions: 1,2.

Definitive hosts: Myctophiformes: Chlorophthalmidae: *Bathypterois ater* (1), *B. dubius* (2).

Distribution: SW Indian Ocean (Mozambique Channel (1)), NE Atlantic (off SW Ireland (2), off NW Scotland (2)).

Discussion

The overall morphology of this species indicates its affinity with *Steringophorus*. Its cirrus-sac and contents may be somewhat atypical in that the seminal vesicle sometimes appears convoluted, saccular, or it may appear to be bipartite or tripartite. In fact, as the cirrus-sac flexes and runs antero-dorsally in its distal region but parallel to the ventral surface proximally, interpretation of its contents is not straightforward, even from sections. In addition, the ventral sucker has a propensity to become withdrawn into the body, pulling the cirrus-sac over onto its antero-sinistral surface. In this condition, which is not dependent on fixation method, the ventral sucker opens into a deep chamber, which in turn opens onto the ventral surface beside the genital atrium, as a narrow aperture bordered posterodorsally by arcs of strong musculature. This is an occasional arrangement; usually the ventral sucker opens directly onto the ventral surface and the genital pore, which lies antero-sinistrally to the ventral sucker, is surrounded by strong superficial muscles, some of which form a distinct patch of diagonally arranged muscle bundles just anterior to the genital pore.

There seems little doubt that the present specimens are conspecific with those described as *Occultacetabulum dorsolineatum* by Reimer (1985), and they share a host genus. The withdrawal of the ventral sucker into the body occurs once in our specimens, but in both specimens lent by Dr. Reimer. The ovary is described as entire by Reimer (1985) and the vitellarium passes further posteriorly. In the present specimens the ovary is lobate, but weakly so, and in one of Reimer's two specimens it is similar, while in the other the ovary appears entire. In Reimer's specimens the vitellarium tends to reach slightly more posteriorly than in the present material. One of Reimer's specimens lacks its anterior end, but the measurements taken from the other give a sucker ratio of 1:0.77. This

Table 1. Measurements of *Steringophorus thulini*.

	Host				
	Coryphaenoides (*Chalinura*) *mediterraneus* (*n* = 5)	*Coryphaenoides* (*Chalinura*) *brevibarbis* (*n* = 1)	*Coryphaenoides* (*Chalinura*) *leptolepis* (*n* = 4)	*Coryphaenoides* (*Chalinura*) *profundicolus* (*n* = 2)	*Trachyrincus murrayi* (*n* = 1)
Length (*L*)	1984–3600	2130	1472–3740	2460–2800	1008
m	2647		2868		
Width (*W*)	784–1264	896	704–1570	970–1020	386
m	1014		1221		
W as % of *L*	32–44 (39)	42	33–51 (43)	36–39	38
Forebody	688–1075	647	552–880	650–670	415
m	848		766		
FB as % of *L*	30–37 (33)	30	22–35 (28)	24–26	41
Oral sucker					
L	231–298	232	238–336	210–220	135
w	219–290	232	245–368	235	146
m	267 × 264		288 × 294		
Ventral sucker					
L	335–451	383	361–528	315–325	191
w	277–367	335	341–578	310–330	197
m	377 × 326		425 × 451		
Sucker ratio	1:1.17–1.27	1:1.44	1:1.32–1.69	1:1.32–1.40	1:1.33
m	1:1.23		1:1.53		
Pharynx					
L	122–136	129	103–161	100–110	70
w	126–149	135	111–167	105–115	72
m	130 × 138		134 × 139		
Oesophagus					
L	187–261	?	70–315	140–200	95
m	222		201		
IB to VS	258–415	?	129–285	195–225	122
m	353		241		
Cirrus-sac					
L	219–338	200	193–330	240–270	107
w	148–193	142	135–232	135–140	79
m	280 × 175		272 × 188		
Ovary					
L	264–399	251	270–600	335–390	?
w	245–328	193	206–405	230–285	
m	328 × 281		414 × 328		
Testes					
L	193–528	229–283	193–595	350–470	127–143
w	225–438	161–187	219–530	265–355	154–156
m	354 × 335		413 × 357		
PTR					
L	586–1296	580	483–1220	800–930	264
m	874		949		
PTR as % of *L*	30–36 (32)	27	33–34 (33)	33	26
Eggs					
L	41–44	42–43	45–52	38–45	Immature
w	17–22	25–27	16–20	20–26	
m	42 × 20		48 × 18	41 × 22	

Note: *L*, length; *w*, width; *m*, mean (length × width); FB, forebody; IB, intestinal bifurcation; PTR, posttesticular region; VS, ventral sucker.

must be treated with caution as the ventral sucker is grossly distorted by its withdrawal into the body. The egg size given by Reimer is slightly smaller than in the present specimens, but my measurements of Reimer's specimens (46–48 × 22–29) show no distinction.

Previously, I (Bray 1988) indicated that I thought that *Occultacetabulum* might belong in the fellodistomine subfamily Monodhelminthinae, but later we (Cribb and Bray 1994) examined specimens and were able to state that the genus did not belong in the Tandanicolinae (syn: Monodhelminthinae). I can now confirm that it belongs in the subfamily Fellodistominae, and I consider it to be a synonym of *Steringophorus*.

***Steringophorus thulini* Bray and Gibson, 1980**

Steringophorus thulini Bray and Gibson, 1980: 226, Fig. 7.
Callodistomoides foliatus: Zubchenko, 1975: 236, Fig. 4 (*nec* Yamaguti, 1970: 42)-Bray, 1983: 333.

Material studied

ex *Coryphaenoides* (*Chalinura*) *mediterraneus* (Giglioli), Gadiformes: Macrouridae. Intestine. Rockall Trough (56°44′N, 09°45′W, depth 1745–1760 m, 19.11.1991. Collector R.A. Bray), BM(NH) 1992.3.24.6–9.

ex *Coryphaenoides* (*Chalinura*) *brevibarbis* (Goode and Bean), Gadiformes: Macrouridae. Intestine. Rockall Trough (57°18′N, 10°24′W, depth 2220 m, 19.11.1991. Collector R.A. Bray). BM(NH) 1992.3.24.16.

ex *Coryphaenoides* (*Chalinura*) *leptolepis* Günther, Gadiformes: Macrouridae. Intestine. Porcupine Abyssal Plain (48°42′N, 16°39′W, depth 4865 m, 11.9.1989. Collector R.A. Bray), BM(NH) 1990.6.14.11–12; Rockall Trough (54°59′N, 12°08′W, depth 2880 m, 18.2.1991. Collector M.E. Spencer Jones). BM(NH) 1992.7.28.12–13; Goban Spur (50°10′N, 14°53′W, depth 4100 m, 5-6.8.1992. Collector S, Mitchell). BM(NH) 1993.1.21.48.

ex *Coryphaenoides* (*Chalinura*) *profundicolus* (Nybelin), Gadiformes: Macrouridae. Intestine. Porcupine Abyssal Plain (48°48′N, 16°31′W, depth 4838–4854 m, 7.9.1989. Collector R.A. Bray). BM(NH) 1990.6.14.13–14.

ex *Trachyrinchus murrayi* Günther, Gadiformes, Macrouridae. ?Stomach. Rockall Trough (56°34′N, 09°31′W, 1310–1445 m, 21.11.1991. Collector M.E. Spencer Jones). BM(NH) 1992.7.28.34.

Description

See Table 1 for measurements. These worms are in accordance with the description by Bray and Gibson (1980: 226), although the new data widen the variation found in some features.

Records: 1. Zubchenko (1975); 2. Bray and Gibson (1980); 3. Present study.

Descriptions: 1,2,3.

Definitive hosts: Gadiformes: Gadidae: *Enchelyopus* (=*Rhinonemus*) *cimbrius* (2), *Gaidropsarus* (=*Onogadus*) *argentatus* (2); Macrouridae: *Coryphaenoides* (*Chalinura*) *brevibarbis* (3), *C.* (*Chal.*) *leptolepis* (3), *C.* (*Chal.*) *mediterraneus*, (3), *C.* (*Chal.*) *profundicolus* (3), *Macrourus berglax* (1), *Trachyrincus murrayi* (3), *T. scabrus* (syn. *trachyrincus*) (2).

Distribution: NE Atlantic [off NW Scotland (2), Skagerrak (2), Rockall Trough (3), Porcupine Abyssal Plain (3), Goban Spur (3),] 'N Atlantic' (1).

Discussion

Steringophorus thulini appears to have a predilection for macrourid gadiforms but also occurs in gadiids in deeper water. It occurs over a wide depth profile, down to abyssal regions, being the first species of *Steringophorus* known to have such a distribution.

***Steringophorus blackeri* Bray, 1973**

Steringophorus blackeri Bray, 1973: 174, Fig. 10-Bray and Gibson, 1980: 220, Fig. 5.

Material studied

ex *Xenodermichthys copei* (Gill), Salmoniformes: Alepocephalidae. Intestine. Goban Spur (49°35′N, 11°50′W, depth 1200 m, 14–15.8.1992. Collector S. Mitchell). BM(NH) 1993.1.21.47.

Comments

The previous five records of this species were listed by Bray and Campbell (1995). Evidence suggests that the species is oioxenic to *X. copei* and is reported from both eastern and western north Atlantic, and deep waters off the northwestern African coast and the mid-Atlantic ridge.

Acknowledgements

My thanks are due to the following: Mary Spencer Jones and Sue Mitchell for collecting some of the specimens; the NERC staff and crews of the RRS *Discovery* and RRS *Challenger* for help and kindness on various cruises; Dr. L. Reimer, Güstrow, Germany, for the loan of specimens; Dave Cooper for the preparation of serial sections; and David Gibson for reading the manuscript.

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Evaluation of characters of the digenean family Gymnophallidae Morozov, 1955

Hilda Lei Ching

Abstract: Characters of the family Gymnophallidae are defined and evaluated to provide a better framework for cladistic analysis and a basis for determining its relationship with other families within the Strigeiformes. Gymnophallids are exclusively marine parasites that commonly use bivalve mollusks and rarely utilize gastropods and polychaetes as intermediate hosts. Marine birds are the final hosts, but human infections have recently been reported in Korea. Daughter sporocysts, cercarial and metacercarial germinal sacs, cercariae, and adults are stages of the life cycle. Depending on the season and species, furcocercous cercariae remain in the sporocyst or leave to find other bivalve or polychaete hosts. The metacercaria are found unencysted and in specific sites where they may cause pits in the shell, concretions, or pearls. Fossilized clams with pits are the first geological evidence of parasitism between digeneans and their hosts. Chick chorioallantoic membrane incubation of the metacercariae combined with scanning electron microscopy of adult gymnophallids can be useful in diagnostics. Seven genera of the family Gymnophallidae can be separated on the basis of 11 characters.

Résumé : Nous définissons et évaluons les caractères de la famille Gymnophallidae afin d'établir un meilleur cadre pour l'analyse cladistique et une base permettant de déterminer la relation de cette famille avec d'autres familles parmi les Strigeiformes. Les Gymnophallidae sont des parasites exclusivement marins qui utilisent en général les mollusques bivalves comme hôtes intermédiaires et, plus rarement, les gastropodes et les polychètes. Les hôtes définitifs sont des oiseaux de mer, mais on a récemment signalé des cas d'infestations chez les humains en Corée. Les sporocystes-filles, les masses germinales des cercaires et des métacercaires, les cercaires et les adultes constituent les stades du cycle de vie. Selon la saison et l'espèce, les furcocercaires restent dans les sporocystes ou en sortent pour trouver d'autres hôtes chez les bivalves ou les polychètes. Les métacercaires ne s'enkystent pas et se retrouvent dans des endroits spécifiques où elles peuvent causer des trous dans la coquille, des concrétions ou des perles. La découverte de coquilles trouées de bivalves fouisseurs fossilisés constitue la première preuve géologique de parasitisme entre les digéniens et leurs hôtes. L'incubation des métacercaires sur membrane chorio-allantoïde de poussin, combinée à la microscopie à balayage des Gymnophallidae adultes, peut être utile pour le diagnostic. À partir de 11 caractères, on peut différencier sept genres de la famille Gymnophallidae.

[Traduit par la Rédaction]

Introduction

Pearson (1992) criticized the many inconsistent characters and confusing terminology used by Brooks et al. (1985) in their cladistic classification of the Digenea. Pearson provided a re-analysis of characters that he believed better reflected the position of the family Heronimidae on the phylogenetic tree. He identified the need for better data from the flukes themselves for a sound cladistic analysis. In Brooks' et al. (1985) classification of the Digenea, the family Gymnophallidae and superfamily Gymnophalloidea were placed in the order Strigeiformes. The diagnoses of the family, superfamily, and order suffer from the lack of accurate and precise data for which Pearson indicated a need. The purpose of this paper is to review and analyze the characters used in the diagnosis of the family Gymnophallidae. I provide information to show that while gymnophallids may have some characters uniquely different from other digeneans, they are not at all complex in their anatomy or life cycles.

Received December 6, 1993. Accepted March 29, 1994. J12490

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Analysis of characters

The discussion on the characters of gymnophallids is based on the historical review of two genera by Stunkard and Uzman (1958), a taxonomic review of the adults by James (1964), a review of the literature including species and host table by Loos-Frank (1971), and studies of gymnophallids by Ching (1965, 1972, 1973*a*, 1973*b*), Pekkarinen (1984,

1986, 1987*a*, 1987*b*, 1987*c*, 1988), and Pekkarinen and Ching (1994). Characters are described and numbered in the order given by Pearson (1992); the numbers of the characters provided by Brooks et al. (1985) are in parentheses.

Miracidium

Few life cycles of digeneans have been completely determined and lack of observations for this stage in this family is not an exception. None of the plesiomorph characters of the miracidium such as the number of flame cells (2, 108), ciliary coverings, presence of eye spots, 19, hatching from the egg and swimming to the snail (1), or hatching after ingestion by the molluscan host (175) should be assumed for this family.

Germinal sac

This character was listed under the heading of 'Larval' by Brooks et al. (1985), which is clearly a wide interpretation of an asexual reproductive stage. The term "primary germinal sac" was used for a miracidium–mother sporocyst or the daughter sporocyst generation of *Parvatrema homoeotecnum* James, 1964 by James (1964). According to James, the "cuticular" envelope surrounding the primary germinal sac may represent the mother sporocyst (3). However, Cable (1982) interpreted the covering around the primary germinal sac of *P. homoeotecnum* to be a thin envelope surrounding, perhaps, a young gymnophallid metacercaria. Pearson (1992) correctly concluded that Ching (1982) used the term "germinal sac" to include stages with the morphology of cercariae or metacercariae that reproduce in the manner of sporocysts and rediae. The gymnophallid germinal sac has embryos, a digestive system similar to that of the cercaria and metacercaria, a ventral sucker, and may have a forked tail. "Germinal sacs reported in some descriptions (140)" by Brooks et al. (1985) refer only to three gymnophallids. Two have a cercarial germinal sac stage: *P. homoeotecnum*, found in the digestive gland and gonad of *Littorina saxatilis* and *Cercaria margaritensis* Ching, 1982, found in the extrapallial cavity of *Margarites* species. *Gymnophallus australis* Szidat, 1962 has a metacercarial germinal sac stage found in *Mytilus platensis*.

Sporocyst

Gymnophallids other than the three above have daughter sporocysts that produce cercariae. Daughter sporocysts are sac-like (3) rather than branching and represent the second (sporocyst) generation (118, 199), and there is an assumption of two generations. A redia stage was reported from a single specimen among the metacercarial germinal sacs of *G. australis* by Szidat (1962), but since no other redia has been found in other gymnophallid life cycles, redia absent (117) may be appropriate.

Cercaria

Pearson discussed "primary excretory pores" as the first character for this stage. Because gymophallid cercariae have forked tails, the position of the excretory pores may be concluded as in the diagnosis of the order to be "at tips of furcae" (111). However, in 10 of 22 descriptions of cercariae, the excretory pores are subterminal to the furcal tips, while in eight they are terminal. Some figures of cercariae do not show the termination of the excretory pores at the furcae. Termination of the excretory pores, which may possibly include "at middle of furcae" (163), may be a useful distinction to separate taxa and should be included in future descriptions.

Pearson (1992) pointed out the discrepancies among characters in the classification of furcocercous cercariae. Cable (1953) described the gymnophallids as belonging to the "Dichotoma group of furcocercous cercariae distinguished by the shape of the excretory vesicle and intestine, absence of flame cells in the tail stem, and distribution in marine lamellibranchs." In descriptions of 21 gymnophallid cercariae, 17 can be classified as dichotoma (139), with tails having furcae and stems equal in size. Two are brevifurcate (110) with short furcae, and one could be classified as longifurcate (135), the latter regarded as a character for the superfamily. Brooks et al. (1985) included cercariaeum with dichotoma (139), but since the former has no tail, it should not be included in this category. To add confusion, some gymnophallid metacercariae found in the extrapallial cavity or mantle of their molluscan hosts were reported mistakenly as "cercariaea." Categories do exist for cercariae without tails, "cercarial tail absent (219, 226)." Four gymnophallid cercariae may lose their tails and become metacercariae within the sporocyst: *Gymnophallus choledochus* Odhner, 1900; *Cercaria discursata* Sinitzin, 1911; *Meiogymnophallus affinis* (Jameson and Nicoll, 1913); and *Cercaria fullbrighti* Hutton, 1952. *Cercaria granosa* Holliman, 1961 described from the sporocyst does not appear to have a furcocercous stage.

Although the size of the excretory bladder is described as large (138) and only refers to gymnophallid cercariae, some renicolid and fellodistomid cercariae have equally large excretory bladders. Perhaps the term 'extensive' is of value when defined in these terms, that is, reaching to mid or anterior to midbody. Of 20 gymnophallid cercariae, 14 have excretory vesicles reaching anterior to the midbody. The excretory bladders are V or Y shaped and filled with concretions that appear black when viewed through a light microscope. The lining of the excretory bladder is thin, or anepitheliocystid (7). The collecting ducts, located at the midbody, are very short with ciliated patches (24) and therefore, the excretory system is mesostomate (107). Other characters of the gymnophallid cercaria are as follows: spinous tegument, distomate (44) or oral sucker (29), ventral sucker (28) present; pharyngeate (6), with bifurcate intestine (12). Two types of glands are present around the oral sucker, and lateral to the pharynx along the ceca. Sensory papillae with or without cilia are found on the tegument; microvilli and sensory papillae are often present on the suckers (Pekkarinen 1988; Pekkarinen and Ching 1994).

Although bioculate cercariae (22) are characteristic of the order, they are not found in members of the Gymnophalloidea. Eye spots were described for *Cercaria margaritae* Lebour, 1908, a synonym of *Meiogymnophallus minutus* (Cobbold, 1859), and for *Gymnophalloides tokiensis* Fujita, 1925, characters refuted for the former by Bowers and James (1967) and for the latter by Ching (1972).

Hosts

The diagnosis of the family by Brooks et al. (1985) is misleading in naming first marine prosobranch gastropods,

and then bivalves as intermediate hosts. Only two gastropod genera have been reported as hosts for the two species with germinal sacs. At least 20 bivalve mollusks are first intermediate hosts for 21 gymnophallid taxa.

Daughter sporocysts occur in the digestive gland or gonad of bivalves. In heavy infections of *Meiogymnophallus affinis* (Jameson and Nicoll, 1913) the sporocysts cause parasitic castration but are found in all of the tissues of the clam *Macoma balthica* (Ching 1965; Pekkarinen 1987*a*).

In the family description by Brooks et al. (1985), gymnophallids are parasitic, "remaining in host or crawling to another pelecypod." Only two cercariae are creepers or poor swimmers and most of the furcocercous cercariae swim actively. Holliman (1961) describes the swimming pattern of *Cercaria imbecilla* Holliman, 1961: "... the tail lashes feebly from side to side with the body flexed ventrally and hanging downward. Periods of swimming alternate with creeping on the bottom with the furcae held together." Such behavior does not preclude passively being taken into the incurrent circulation of potential bivalve hosts, but creeping behavior towards potential hosts has not been recorded.

Metacercaria

In the Strigeiformes, the cercariae "encyst" in the next host (106) but gymnophallid cercariae do not encyst (11) and some remain in the invertebrate host until ingested by a vertebrate host. Cable (1953) stated for the gymnophallids: "metacercaria unencysted in mollusks although often embedded in a mucoid substance or enclosed in capsules of host origin and stimulating pearl formation." According to their original descriptions, mucoid envelopes are present for *Metacercaria megacoela* Palombi, 1934; *Parvatrema borinquenae*, Cable, 1953, *Parvatrema donacis* Hopkins, 1958; and *Parvatrema timondavidi* Bartoli, 1963.

Host sites and pathology

Gymnophallid metacercariae may be found free in the extrapallial cavity, embedded in the mantle epithelium, lying against the extrapallial line of the shell, or within the adductor muscles of the clam hosts. Each species is found in a specific site within the clam host. Multiple infections with gymnophallid metacercariae are possible with each species occupying its own microhabitat (Lauckner 1983). For example, *Meiogymnophallus fossarum* Bartoli, 1965 is found in two sites in *Cardium glaucum*. When the parasite is located between the shell and mantle below the hinge, no damage is caused. However, metacercariae located at the periphery of the pallial line can cause gaping and asymmetry of the valves, and disorientation of the clam hosts. In mixed infections of *M. fossarum* and *Meigymnophallus minutus* in *Cerastoderma edule*, *M. fossarum* occurred free at the hinge or along the pallial line, while *M. minutus* was enclosed in tissue at the hinge (Russall-Pinto and Bartoli 1992). Similarly, the metacercaria of *Gymnophallus somateriae* (Levinsen, 1881) was found in the adductor muscles, causing concretions or pearls, while *Lacunovermis macomae* (Lebour, 1908) was found within pits in the shell near the hinge of *Macoma balthica* (Ching 1965; Pekkarinen 1988). The presence of pits in fossil clams was used to demonstrate the first geological record of parasitism between digeneans and their bivalve hosts. By examining the pits in the shells of fossil *Gemma*, *Parastarte*, and *Transennella*, Ruiz and Lindberg (1989) showed that the association of *Parvatrema borealis* with its clam hosts has existed for at least 5 million years.

As well as pathological effects, gymnophallids may change their hosts' orientation and behavior. *Venerupis aurea* infected with the metacercariae of *M. fossarum* were more visible in the sand substrate than nonparasitized clams, probably facilitating predation by oystercatchers (Bartoli 1978). Gymnophallids affected the distribution of their hosts, *Macoma balthica*, so that more metacercariae were found in clams at higher tidal levels, in larger rather than smaller clams, and in more crawlers than in buried clams (Lin and Green 1991).

Bivalves serve in most cases as the second intermediate hosts for gymnophallid metacercariae (Loos-Frank 1971). Reports of *Gymnophalloides tokiensis* in oysters in Japan and *Parvatrema timondavidi* in *Tapes philippinarum*, a commercially important clam in Korea, indicate a potential for human infection if the infected bivalves are eaten raw or lightly cooked (Yu et al. 1993). Only five gastropods, six polychaetes, and one brachiopod have been reported as second intermediate hosts, the last by Paine (1962). The metacercariae found in gastropod hosts are *Cercaria glandosa* Lebour, 1908 from *Hydrobia stagnalis* and *Parvatrema* sp. 2 Rebecq, 1964 from *Hydrobia ventrosa* according to Loos-Frank (1971); *Parvatrema obscurum* (Ching, 1960) from *Lottia digitalis* and *Collisella scabra* reported by Ching and Grosholz (1988), and *Parvatrema* species in *Melampus bidentatus* reported by Heard (1970). Four metacercariae including one unidentified species have been reported in or on polychaetes (Margolis 1971, 1973; Kyle and Noble 1985). Bartoli (1972) reported that polychaetes were the only second intermediate hosts for *Gymnophallus nereicola* Rebecq and Prevot, 1962, but they and bivalves are both second intermediate hosts in the life cycles of *Parvatrema borealis* Stunkard and Uzman, 1958 and *G. choledochus* as described by Loos-Frank (1969).

Anatomy

Although well developed in the metacercarial stage, the gymnophallid reproductive system will be described later for adults. The gymnophallid excretory system is well developed but hasn't been compared with related groups because of the variable shape of the excretory vesicle. The excretory bladder is basically V or Y shaped but the "V" may have a thin or thick point and the stem of the "Y" may also be thin or broad with dorsal extensions or lobes. The lateral arms may be bicornuate or lobate. The excretory bladder in the gymnophallid cercaria continues to increase in extent and size in the metacercaria. For instance, in 4 months of experimental development, the excretory vesicle of *Gymnophallus fossarum* is Y shaped with lobes, but 2 months later, the bladder increases in size and extent so that all of the organs except for the ventral sucker are obscured (Bartoli 1972). Concretions in the excretory bladder may differ in size and be diagnostic for each species (Pekkarinen 1988). Typically, the excretory system has a short collecting duct arising from the side of each lateral arm, with flame cells branching from the anterior and posterior

tubules in patterns of two, totalling 16 or 24. Thus the short collecting tubules continue to be mesostomate in older gymnophallids.

The digestive system is also well developed with an oral sucker, pharynx, and short ceca. The oral sucker often has lateral extensions called "lateral lips" or "lateral papillae" and is surrounded by sensory papillae. In *Gymnophallus rostratus* Bartoli, 1982, a rostrum anterior to the oral sucker contains openings of the cephalic glands. The digestive ceca, usually found only in the anterior half of the body, are lined with large cells and sometimes filled with pigmented or granular material.

The spines on the tegument of the metacercaria may change in shape from sharp to broad and serrated (Pekkarinen and Ching 1994).

Adult

Characteristics that may be descriptive for the order but which are not for most gymnophallids are ovary between testes (112), genital pore in mid-hindbody (113), and uterus extending anteriorly from ovary to near acetabulum posteriorly to genital pore (114). A spinose cirrus (136) is not present and is not a character for the superfamily.

Descriptive characters of adults in the family Gymnophallidae

Small to minute Strigeiformes. Body oval to pyriform. Tegument spined (47) with simple, serrated, or sharply ridged spines within pits. Body spines arranged in transverse tiers or obliquely arranged (quincunx), differing in size along dorsal and ventral surfaces. Spines may be lacking in small patches on the dorsal and ventral surfaces. Oral sucker present (14), larger, sometimes twice the size of ventral sucker, with lateral papillae (231) or without. Sensory papillae arranged in a pattern on oral sucker. Ventral sucker present (16), located in middle third of body, with sensory papillae. Pharynx near oral sucker (17). Esophagus very short. Intestine bifurcate (15), ceca widely divergent, often enlarged, short (28, 225), not reaching to midbody, with or without dorsal extensions. Ventral pit present or absent, anterior to ventral sucker. Testes two, 35, round or ovoid, 36, symmetrically or obliquely arranged to each other, located posterolateral to ventral sucker, 37. Seminal vesicle present, undivided, bi- or tri-partite. Pars prostatica present, 44, well developed with tubular or oval vesicle, or absent. If pars prostatica absent, gland cells surround genital atrium. Cirrus sac not present (137), cirrus absent. Ejaculatory duct and metraterm join to form gonoduct. Genital atrium tubular, or transversely oval (53). Genital pore medial and postbifurcal (51), type A2 in Pearson's (1992) diagram, immediately anterior, on the edge, or some distance anterior to ventral sucker, never in hindbody. Genital pore wide and pit-like or small, inconspicuous, with or without sensory papillae. Ovary rounded or ovoid (45), anterior to left or right testis (46), rarely intertesticular. Laurer's canal present (56). Mehlis gland well developed; seminal receptacle or fertilization chamber present (171). Vitelline glands varying in shape from paired group of follicles (226) to paired compact (93) to single lobed or compact organ usually located anterior, lateral or directly

Table 1. Key characters of the family Gymnophallidae Morozov, 1955.

Genus:	*Gymnophallus* Odhner, 1900	*Gymnophalloides* Fujita, 1925	*Parvatrema* Cable, 1953	*Meiogymnophallus* Ching, 1965	*Lacunovermis* Ching, 1965	*Paragymnophallus* Ching, 1973	*Pseudogymnophallus* Hoberg, 1981
Type species:	*deliciosus*	*tokiensis*	*borinquenae*	*affinis*	*macomae*	*odhneri*	*alcae*
Characteristic							
Body spines	Sharp	Broad	Broad	Broad, serrated	Broad, serrated	Broad	Broad, scaly
Lateral papillae of oral sucker	Absent	Present	Present	Present	Present	Absent	Absent
Ventral pit	Absent	Present	Absent	Absent	Present	Absent	Absent
Cecal pockets	Absent	Absent	Absent	Absent	Absent	Absent	Present
Seminal vesicle	Bi-,tri-partite	Bipartite	Undivided	Undivided	Undivided	Bipartite	Undivided
Prostatic duct	Elongate	Elongate	Oval	Oval	Oval	Oval	Elongate
Genital pore, papillae	Small, absent	Small, absent	Wide, present	Small, present	Wide, present	Wide, absent	Small, absent
Genital atrium	Tubular	Tubular	Wide, oval	Tubular	Wide, oval	Tubular	Tubular
Vitellaria	Follicular	Compact	Compact	Compact, lobed	Compact	Follicular	Follicular
Excretory vesicle	Y shaped	V shaped	V shaped	V shaped	V shaped	V shaped	Y shaped
Uterus extent	Fore, hindbody	Forebody	Fore, hindbody	Fore, hindbody	Hindbody	Hindbody	Hindbody

posterior to ventral sucker. Uterus passing postovarian then anteriorly to cecal bifurcation (16), in forebody, hindbody, or entire body. Eggs less than 40 μm in length (174), round in section (48), operculate (49), embryonated when laid. Excretory vesicle V or Y shaped with simple lateral arms (54); arms extending forward to pharynx, with or without diverticula. Excretory collecting duct short, with cilia. Collecting tubules mesostomate. Flame cells in patterns of two with total of 16 or 24. Excretory pore terminal (55). Eleven characters that separate seven genera are listed in Table 1.

Location

Gall bladder, bursa Fabricii, intestine of marine birds such as gulls, diving ducks, shorebirds. One mammalian infection reported, that of *Gymnophalloides seioi* Lee, Chai and Hong, 1993 in the intestine of humans, Korea (Lee et al. 1993).

Life cycles

Loos-Frank (1969) categorized three types of gymnophallid life cycles. I*a*. Free swimming cercaria and metacercaria occur in two intermediate hosts, e. g., *P. borinquenae* Cable, 1953 in *Gemma purpurea* and *Cerithidea costata*. I*b*. Free swimming cercaria and metacercaria occur in individuals of the same species (one host), e. g., *Lacunovermis macomae* in *Macoma balthica*. II. Free swimming cercaria and metacercaria occur in two intermediate hosts in phase of the life cycle alternating seasonally with a cercaria that loses its tail and remains in sporocyst within the same host, e. g., *Gymnophallus choledochus* in *Cardium edule* and *Nereis diversicolor*. III*a*. Cercaria loses its tail, remains in sporocyst within the same host, e. g., *Meiogymnophallus affinis* in *Macoma balthica*. III*b*. Larva remains in germinal sac in same host, e. g., *P. homoeotecnum* in *Littorina saxatilis*.

Gymnophallid life cycles differ from other strigeoid life cycles in that bivalves replace gastropods as the first intermediate host and invertebrates replace vertebrates as the second intermediate host. Typical gymnophallid life cycles involve bivalves as both intermediate hosts, bivalves and polychaetes as second intermediate hosts, or bivalves as single hosts. Gastropods are rarely second intermediate hosts.

Taxonomy

Identifications of gymnophallids have been confusing because the worms are extremely small and ovigerous and the descriptions have been poor. Many cercariae, metacercariae, and adults probably belong to one or two taxa but insufficient life cycle studies or anatomical comparisons have been made to establish this. Identifications have been facilitated with the recovery of adults upon incubation of metacercariae on chick chorioallantoic membrane (CAM) followed by SEM studies. Using these techniques, Pekkarinen and Ching (1994) were able to synonymize the names of three pairs of gymnophallids that parasitize a clam host with wide geographic distribution. Biological data such as the locations in intermediate hosts, ecological niches, and the type of host species are important, particularly when two species such as *G. nereicola* and *G. fossarum* are anatomically similar (Bartoli 1972, 1981).

The present review of the Gymnophallidae indicates the need for an evaluation of the order to better reflect the characters of this family and its relationships with other families within the order. For instance, the family Fellodistomidae, although polyphyletic in origin, is closely related to the gymnophallids but was not discussed at all by Brooks et al. (1985).

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Relationship of parasites (Trematoda: Spirorchidae) and their eggs to the occurrence of fibropapillomas in the green turtle (*Chelonia mydas*)

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Abstract: The prevalence of green turtle (*Chelonia mydas*) fibropapillomatosis is increasing in Hawaii, raising the possibility of long-term negative effects on the breeding population. As the etiology of this disease is unknown, our objective was to determine if any relationship exists between fibropapillomas and trematode eggs from the family Spirorchidae, whose presence in tissue sections has been noted for years. A process for tumor digestion and egg extraction was developed and a total of 61 tumors of all sizes was examined to determine the total numbers of eggs present and the species of spirorchid involved. Twelve samples of normal turtle tissue were also examined. Eggs of one to three spirorchid species (*Leardius learedi* Price, 1934; *Haplotrema dorsopora* Dailey, Fast and Balazs, 1993; and *Carettacola hawaiiensis* Dailey, Fast and Balazs, 1991) were recovered from all tumors and nontumored tissues digested. Five turtles were injected with spirorchid eggs and observed for 12 months. New tumors developed on two of the turtles at noninjected sites. Although attempts to induce fibropapillomas through injection were not successful, the presence of spirorchid eggs in every tumor examined raises additional questions regarding the relationship between these parasites and fibropapillomas in sea turtles.

Résumé : La prévalence de la fibropapillomatose augmente chez la tortue verte (*Chelonia mydas*) à Hawaii, ce qui fait craindre des effets négatifs à long terme sur la population reproductrice. L'étiologie de cette maladie étant inconnue, nous avons tenté de déterminer s'il existait une relation entre les fibropapillomes et les oeufs de trématodes de la famille Spirorchidae, dont la présence est observée dans les coupes de tissus de tortue depuis des années. Nous avons mis au point un procédé de digestion des tumeurs et d'extraction des oeufs et examiné au total 61 tumeurs de diverses tailles pour y déterminer le nombre total d'oeufs présents et les espèces de Spirorchidae en cause. Nous avons aussi examiné 12 échantillons de tissu normal de tortue. Nous avons retrouvé des oeufs de 1 à 3 espèces de Spirorchidae (*Leardius learedi* Price, 1934; *Haplotrema dorsoporal* Dailey, Fast et Balazs, 1993; *Carettacola hawaiiensis* Dailey, Fast et Balazs, 1991) dans toutes les tumeurs et tous les tissus sans tumeurs digérés. Nous avons injecté à cinq tortues des oeufs de Spirorchidae et nous les avons ensuite observées pendant 12 mois. De nouvelles tumeurs sont apparues chez deux des tortues ailleurs qu'au point d'injection. Si nous n'avons pas réussi à faire apparaître des fibropapillomes par l'injection, la présence d'oeufs de Spirorchidae dans chacune des tumeurs soulève de nouvelles questions quant à la relation entre ces parasites et les fibropapillomes chez les tortues de mer.

[Traduit par la Rédaction]

Introduction

Fibropapillomas (GTF) in the green turtle (*Chelonia mydas*) were first described more than 55 years ago from the Florida Keys (Lucke 1938; Smith and Coats 1938). Little work on this disease was done during the next 40 years. Beginning in 1982, cutaneous fibropapillomas were seen in green turtles in the Indian River Lagoon system in east central Florida, and between 1985 and 1986, 57% of green turtles collected from this locality were infected (Ehrhart 1991). During 1985, 35% of the 51 stranded green turtles examined throughout the Hawaiian Islands had fibropapillomas (Balazs 1986), the growths ranging from a few millimetres to more than 30 cm in diameter (Dailey and

Received February 24, 1994. Accepted December 15, 1994
J12519

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Table 1. Summary of turtles sampled.

Turtle No.	Collection locality	Date*	Weight (kg)	Carapace length (cm)	Sex
1	Oahu	3/2/92	18.6	58.8	F
2	Oahu	1/8/92	20.0	53.4	F
3	Maui	4/13/92	11.8	47.8	M
4	Oahu	8/11/92	21.4	56.7	F
5	Oahu	6/16/92	17.7	48.2	—
6	Oahu	6/16/92	41.8	67.3	—
7	Oahu	6/10/92	20.9	56.9	F
8	Oahu	9/22/92	18.2	56.0	F
9	Oahu	9/22/92	43.6	71.3	M

*Month/day/year.

Balazs 1987). These growths in turtles can result in reduced vision, disorientation, blindness, and physical impairment of normal swimming, feeding, and breeding.

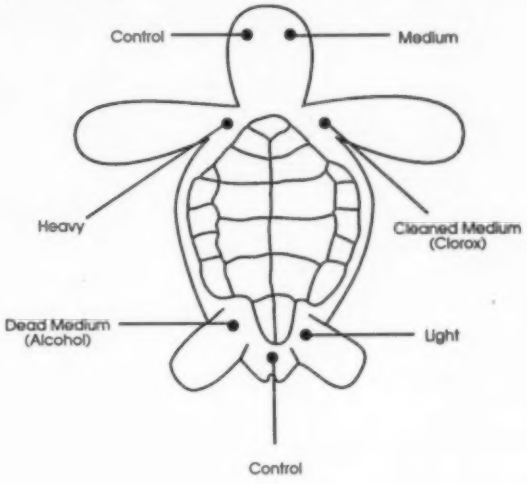
From 1986 to 1988, 10 green sea turtles from the Hawaiian islands of Lanai, Maui, and Oahu were surveyed for parasites and tumors. The results of this survey indicated that Hawaiian green turtles were heavily parasitized and that their tumors contained large numbers of trematode eggs from the family Spirorchidae (Dailey et al. 1991, 1992, 1993). The etiologic agent for these fibropapilloma tumors is still the object of speculation. One suggested theory is a parasite–tumor relationship. The present study was undertaken to look at this relationship by quantifying the size and location of all tumors examined and by noting egg type and number per tumor.

Materials and methods

Turtles were hand-captured from Kaneohe Bay, Oahu, Hawaii, by personnel of the National Marine Fisheries Service and the tumors measured and excised prior to the animal's release. Turtles found stranded on shore and which demonstrated no chance for survival were killed for necropsy using pentobarbital sodium (Table 1).

Tumors were surgically removed from all turtles. Live turtles received a lidocaine nerve block before tumor removal. Most of the live turtle samples consisted of small tumors and the animals did not require sutures after their removal. All tumors were measured and weighed prior to digestion processing. Large tumors from freshly killed turtles were cut into smaller pieces to facilitate digestion. Tumors were digested in 1 : 10 000 pepsin from porcine stomach mucosa. Approximately 9.5–10.0 g pepsin was mixed with 50 mL of water for the digestive process, giving a concentration that was deemed adequate for the digestion through trial and error. The tumor tissue was placed in plastic containers with perforated lids and incubated at 40°C for 48–72 h. After digestion, eggs were extracted by passing through a Flukfinder. The Flukfinder (trade mark name by Visual Difference, 5051-C Old Pullman Rd., Moscow, ID 83843) consists of a double filtration container with stacked top and bottom stainless steel screens of 100 and 40 μm, respectively. Eggs trapped by the bottom

Fig. 1. Injection sites on experimental green turtles (ventral view).



screen were backwashed into a beaker using a squeeze bottle. The collected material was then centrifuged for 5 min at 1550 rpm. The residue was then fixed with several drops of 10% formalin and stored until it could be examined using a compound microscope for total number of eggs and species represented. Eggs per gram weight of tissue was calculated. For control samples, normal tissue was collected by punch biopsy and processed in the same manner as tumor material.

Usually, most digestion was complete in 48 h except for some remaining fibrous tissue. Occasionally, some larger pieces had to be incubated for longer periods. Incubation times of up to 4 d did not affect the egg extraction procedure. Spirorchid eggs were incubated and observed for 5 d in this solution with no apparent effect.

Five turtles captured from Kaneohe Bay were selected for experimental infection (Table 2). Four of the turtles were free of any visible external tumors, while the fifth had numerous tumors present. The visibly tumored turtle was selected as part of the study to determine whether turtles with growths are sensitized by the infection and perhaps more prone to develop new growths. The turtles were maintained on a diet of previously frozen squid with vitamin–mineral supplements at the National Marine Fisheries Service's Kewalo Research Facility in Honolulu.

A source of viable spirorchid eggs was found in the serosal vessels of the small intestine of necropsied tumored turtles. Each blocked vessel contained a mass of 100–150 eggs. Viability was confirmed by observing miracidial activity (contractions) within the egg. Eggs maintained in sea water at ambient temperature (25°C) remained viable to 30 d. Eggs were injected subcutaneously in the same sites on all turtles (Fig. 1). The injections were divided into three dosages of eggs suspended in saline (0.2 mL): light (100–150), medium (300–500), and heavy (1000–1500), which were injected into the base of the right rear flipper site, the left lower eyelid site, and the base of the right front flipper site. The base of left rear

Table 2. Length and weight gains of turtles following experimental exposure to infection.

	Initial		12 month period			
Turtle	Carapace length (cm)	Weight (kg)	Carapace length (cm)	Gain	Weight (kg)	Gain
H924	42.2	10.0	51.9	+9.7	20.5	+10.5
V471	42.2	8.6	50.0	+7.8	18.2	+9.6
H951	41.7	10.9	46.9	+5.2	16.4	+5.5
V393	40.7	10.0	45.6	+4.9	14.5	+4.5
H921*	42.9	11.8	46.0	+3.1	15.0	+3.2

*Indicates turtle having visible external tumors.

Table 3. Number of spirorchid eggs found in tumors of green turtles 1, 2, 7, 8, 9.

Site	N*	Average tumor wt. (g)	Average no. of eggs/g by species†			Average total no. of eggs recovered	Average total no. of eggs/g tumor tissue
			Ll	Hd	Ch		
Cornea	3	2.6 (0.5–4.0)	231 (36–631)	82 (36–209)	5 (1–15)	1 127	318
Eye	5	2.4 (0.5–5.5)	41 (29–106)	23 (9–58)	0.8 (0.4–3)	458	170
Jaw	2	1.4 (1.0–1.7)	45 (38–51)	41 (34–47)	4 (3–5)	232	90
Neck	5	9.5 (1.5–20.0)	27 (1.5–78)	35 (4–86)	0.6 (0–1)	3 064	63
Right front flipper	11	47.1 (0.1–180.0)	6 (0.5–32)	33 (1–141)	4.4 (0–14)	18 263	43
Left front flipper	4	79.5 (1.0–137.0)	21 (1.0–5.8)	39 (5–113)	2.3 (0–8)	4 683	83
Right rear flipper	7	1.4 (1.0–3.0)	22 (7.0–490)	42 (19–95)	5.4 (0–13)	767	69
Left rear flipper	5	85.0 (0.6–347.0)	11 (0.8–20)	46 (5–107)	3.6 (0–15)	21 945	61
Cloaca	1	0.5	0	26	0	26	52
Glottis	1	3.2	6	28	0	34	11
Lung	6	6.1 (0.2–13.4)	122 (3–215)	51 (1–204)	2.6 (0–6)	3 988	176
Kidney	3	26.0 (5.5–74.0)	35 (15–51)	68 (11–83)	11 (1–32)	20 653	114
Liver	5	60.0 (1.5–144.0)	7 (2–15)	27 (1–90)	1.8 (0–4)	15 426	36
Total eggs/g by species			568	541	62		
% total eggs/g by species		49	46	5			

*N, number of tumors examined.
†Values are means followed by ranges in parentheses. Ll, *Leardius learedi*; Hd, *Hapalotrema dorsopora*; Ch, *Carettacola hawaiiensis*.

flipper site was injected with alcohol-killed eggs. The base of left front flipper site was injected with eggs disinfected with 10% Clorox to eliminate any microorganisms. Both sites received a medium dose. Two control sites (right lower eyelid, cloaca) were injected with saline solution. One turtle (V471) received an additional (heavy dosage) injection of eggs in saline at the right front flipper tag site.

All turtles were examined for tumor growth and their lengths and weights were measured every 2 weeks for 6 months and monthly thereafter for 1 year.

Results

A total of 61 tumors from both wild-caught and beached, necropsied turtles were digested and examined. These tumors were collected from both internal and external sources and ranged in weight from 0.2 to 347 g. Eggs were recovered from 100% of tumors examined, with egg numbers ranging from less than 1 to more than 845 per gram of tumor tissue. Turtles 1, 2, 7, 8, and 9 were heavily tumored, providing 93% of all material examined (Table 3). Turtle 3 had no external tumors and was included with the nontumored tissue sample (Table 4). Turtles 4, 5, and 6 were capture/release animals, which were used primarily for obtaining very small tumors (Table 5).

Experimental infections were induced in five turtles (Table 2). All experimentally infected turtles demonstrated rapid length and weight gains during the year in captivity. The tumored turtle (H921), however, showed less growth in both areas than the four nontumored turtles. Two turtles (V471, H951) developed new tumor growths that were histologically diagnosed as fibropapillomas. After 3 months, a tumor appeared at the control injection site (right lower eyelid) in V471, which grew to a maximum size of 9 × 4 mm over the 12-month period. During

Table 4. Number of spirorchid eggs found in nontumored tissue of green turtles.

Turtle No.	Site	Wt. (g)	Eggs by species*			Total eggs
			Ll	Hd	Ch	
1	Left front flipper	1.3	0	11	1	12
1	Neck	5.0	1	1	0	2
1	Left rear body	4.0	18	18	0	36
2	Neck	4.5	2	1	0	3
2	Right front flipper	2.5	7	21	0	28
2	Right rear flipper	18	2	2	0	4
3	Neck	113	1	0	0	1
3	Right front flipper	113	0	2	0	2
3	Right rear flipper	79.2	2	6	0	8
7	Kidney	30	496	544	2 224	3 264
7	Heart	77.5	2 650 +	43 999 +	159 +	46 808 +
7	Brain (entire)	4	367	906	0	1 273

*Ll, *Leardius learedi*; Hd, *Hapalotrema dorsopora*; Ch, *Carettacola hawaiiensis*.

Table 5. Number of spirorchid eggs found in tumors of green turtles captured and released.

Turtle No.	Site	Tissue wt. (g)	Eggs by species*			Total eggs	Total eggs/g
			Ll	Hd	Ch		
4	Eye	0.5	8	8	0	16	32
4	Shell	0.6	23	30	0	53	88
5	Right front flipper	0.4	2	0	0	2	5
6	Neck	2.1	8	59	0	67	32
Total eggs by species			41	97	0		
% total eggs by species			30	70	0		

*Ll, *Leardius learedi*; Hd, *Hapalotrema dorsopora*; Ch, *Carettacola hawaiiensis*.

month 11, the same turtle developed another growth in the left eye (medial canthus), which grew to 3 × 2 mm in the final month of observation. The second turtle (H951) also developed a tumor in the same area as V471 (medial canthus of left eye) at the same time (11 mo). This growth measured 4 × 3 mm by end of month 12.

Discussion

The factors (pathogen plus environmental insult) responsible for the development of fibropapillomas in green turtles have yet to be confirmed. Recently, Jacobson et al. (1991) reported the presence of a herpesvirus in two cases of GTF. They were unable, however, to fulfill Koch's postulate and demonstrate that a causal relationship existed between the herpesvirus and the tumors. They found no evidence of herpesvirus infection, as indicated by the presence of intranuclear inclusions, in any of the sectioned material originating from the 73 tumors from Hawaiian green turtles. Jacobson (1992) stated that trematode eggs from the family Spirorchidae were seen more often within the dermal capillaries of cutaneous GTF of Hawaii-caught turtles as compared to those from Florida. He indicated that trematode eggs were few in sections of tumor examined, and that multiple sections of a given tumor may need to be examined in order to clearly demonstrate the presence or absence of eggs.

Pollutant-mediated stress causing morphophysiological changes such as cellular proliferation (i.e., skin tumor) is well documented in aquatic organisms (Giam and Ray 1987). Chemical pollutants impairing the immune system and causing stress have been listed as possible contributors to GTF in Hawaiian green turtles (Aguirre 1991, 1992; Balazs and Pooley 1991). Aguirre (1993), to determine the involvement of selected environmental pollutants on the etiology of GTF, examined 11 heavily tumored turtles from Hawaiian waters. He tested brain, fat, liver, and kidney tissues for any of the listed organochlorines, polychlorinated biphenyls, organophosphates, or carbamate insecticides in concentrations above stated method of detection limits. He also looked at concentrations of selenium and heavy metals. In all cases he found that concentration levels were below levels reported to be normal in other animal species.

The present study deteremined the total number of eggs that could be recovered from the entire tumor through digestion and identified the species of spirorchid trematodes involved and their degree of involvement. The results

indicate clearly that 100% of all tumors found during this study, regardless of size or location, contained spirorchid eggs. Not only did all tumors contain eggs, but the eggs were sometimes present in massive numbers, particularly in tumors located in internal organs (Table 3). A tumor on the cornea of turtle 2 contained 631 eggs/g tissue. Even the smallest tumors contained a significant number of eggs. A 0.6-g shell tumor had 88 eggs/g and a 0.5 g eye tumor had 32 eggs/g. As previously stated, internal tumors also contained large concentrations of ova. For example, a lung tumor weighing 2.5 g contained 243 eggs/g, while the average lung granuloma (internal growth) contained 176 eggs/g. The liver and kidneys were also tumored in some turtles, the liver tumors averaging 36 eggs/g, while kidney tumors were somewhat higher at 114 eggs/g. Massive numbers of eggs were also found in nontumored internal organs and the brain (Table 4). Although the significance of this information has yet to be determined, we feel it strongly suggests a direct link between GTF and spirorchid trematode infections.

Three species of blood fluke, *Leardius learedi* Price, 1934; *Hapalotrema dorsopora* Dailey, Fast and Balazs, 1993; and *Carettacola hawaiiensis* Dailey, Fast and Balazs, 1991, which are found in large numbers in Hawaiian green turtles, occupy niches similar to those occupied by members of the genus *Schistosoma* (i.e., the circulatory system). The finding in this study of a high egg count in brain tissue (318/g in turtle 7) demonstrates the extent to which these eggs are spread in the circulatory system. The effect on the turtle of such large numbers of eggs was examined by Wolke et al. (1982). During their study of loggerhead sea turtles (*Caretta caretta*) along the eastern Atlantic seaboard, they found eggs representing three genera of spirorchid trematodes: *Carettacola*, *Hapalotrema*, and *Neospirorchis*. Gross signs of pathology in heavily infected animals included cachexia, anemia, and enteritis. They also noted a similarity in the histopathological lesions to those present in homeotherms with schistosomiasis. These authors concluded that spirorchidiasis is prevalent in subadult loggerhead sea turtles and that it may be responsible for significant debilitation and mortality.

Although the mode of transmission of marine species of the family Spirorchidae has not been elucidated, the life cycle of *Spirorchis elegans* Stunkard, 1923, a species that lives in the blood vessels of freshwater turtles, is known (Goodchild and Kirk 1960). In the life cycle of *S. elegans*, the cercariae are shed from a snail and penetrate the mucous membranes of the eye, nose, mouth, and cloaca of turtles. Recent studies on snails inhabiting the feeding grounds of Hawaiian green turtles have, to date, not revealed the presence of spirorchid trematode larvae (M.D. Dailey, unpublished data). This observation indicates that the life cycle of marine spirorchids infecting sea turtles may not involve a snail intermediate host. Køie (1982) has found that *Aporocotyle simplex* Odhner, 1900, a sanguinicolid blood fluke of marine fishes, uses a polychaete as the sole intermediate host.

Preliminary work has begun on isolating the protein that causes the host to react to the ova. Findings to date indicate that a protein is present in the sterile saline solution in which eggs recovered from the serosa of tumored turtles have been cultured (Dr. J. Sakanari, School of Medicine, Department of Pathology, University of California, San Francisco, CA 94121, personal communication). Additional work is needed to find the specific protein involved and to confirm that it is parasite rather than host induced. Once isolated, this protein could be collected and tested to determine its role in GTF production.

Acknowledgments

The authors thank George Balazs, National Marine Fisheries Service, Honolulu Laboratory, for his many contributions and guidance throughout this study. This work was funded by NMFS contract No. 40ABNF201243. We gratefully acknowledge this support.

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Allopodocotyle margolisi n.sp. (Digenea: Opecoelidae) from the deep-sea fish *Coryphaenoides* (*Chalinura*) *mediterraneus* in the northeastern Atlantic

David I. Gibson

Abstract: An opecoelid digenean parasite, ***Allopodocotyle margolisi*** n.sp., is described from the macrourid fish *Coryphaenoides* (*Chalinura*) *mediterraneus* from benthopelagic waters of the North Rockall Trough, NE Atlantic Ocean. This is the first member of the genus to be recorded from deep-sea fishes. It is distinguished from its neritic congeners on the basis of features that include the arrangement of the testes, length of the cirrus-sac, and sucker-ratio. Its distinctness from other opecoelids from deep-sea fishes is also commented upon.

Résumé : Le présent article décrit l'Opecoelidé ***Allopodocotyle margolisi*** n.sp., parasite digénien isolé à partir du Macrouridé abyssal *Coryphaenoides* (*Chalinura*) *mediterraneus* dans les eaux benthopélagiques de la dépression bordant le côté nord du plateau Rockall, dans l'Atlantique Nord-Est. L'organisme est le premier représentant du genre associé à des poissons abyssaux. Il diffère de ses congénères néritiques par un certain nombre de caractères, en particulier l'arrangement des testicules, la longueur du cirrus et les ventouses. Les caractères permettant de le différencier des autres Opecoelidés parasitant les poissons abyssaux sont également décrits.
[Traduit par la Rédaction]

Introduction

Despite their complex life-history and transmission problems caused by the relatively low population density of their hosts, digeneans are common parasites of deep-sea benthic fishes. Published records are few, but they include records of digeneans that extend well into the abyssal region, the deepest being that of the lepocreadiid *Profundivermis intercalarius* Bray and Gibson, 1991 from the macrourid *Coryphaenoides* (*Nematonurus*) *armatus* from 4850 m on the Porcupine Abyssal Plain, NE Atlantic (Bray and Gibson 1991). While not nearly as prevalent as lepocreadiids, opecoelid digeneans are an important, but little studied, group of parasites in deep-sea fishes. Although they have been recorded as deep as 3000–3300 m in the form of *Neolebouria merretti* Gibson and Bray, 1982 from the myctophoid *Bathytyphlops sewelli* off the Canary Isles (Gibson and Bray 1982), the majority of opecoelid records in deep-sea fishes are from bathyal waters (usually <1000 m). Macrourids represent the most important group of deep-sea fishes in the lower latitudes. According to the Host–Parasite Data-base at The Natural History Museum, London, opecoelids previously recorded from this group are mainly from North Atlantic and neighbouring waters, and include the opecoeline *Pseudopecoelus tortugae* von Wicklen, 1946 (syn. *Cymbephallus fimbriatus* Linton of Manter 1934) from *Coelorynchus carminatus*, *Hymenocephalus italicus*, and *Ventrifossa occidentalis* (see Manter 1934; Armstrong 1974); and the plagioporines *Tellervotrema armstrongi* Gibson and Bray, 1982 (syn. "*Plagioporus* sp.n. #1 and ?#2" of Armstrong 1974 from *Nezumia aequalis*, see Armstrong 1974; Gibson and Bray 1982), *T. beringi* (Mamaev, 1965) from *Coryphaenoides* sp. in the Bering Sea (see Mamaev 1965), *Podocotyle pearsei* Manter, 1934 from *Bathygadus macrops* and *Gadomus arcuatus* (see Armstrong 1974), *Bathycreadium biscayense* Bray, 1973 from *Trachyrincus trachyrincus* and *Coelorinchus coelorhincus* (see Bray 1973; Orecchia and Paggi 1978), and *B. flexicolis* Kabata, 1961 from *Macrourus berglax* and *Coelorinchus coelorhincus* (see Kabata 1961; Orecchia and Paggi 1978). There are also undescribed forms listed by Campbell et al. (1980) as "*Plagioporus* sp.n." and "*Podocotyle* sp.n." from from *Coryphaenoides* (*Nematonurus*) *armatus* from the northern NW Atlantic and several unpublished species described in the thesis of Armstrong (1974). The latter include two species of "*Podocotyle*" from *Ventrifossa atlantica*, one from *Sphagemacrurus grenadae* and one from *Nezumia aequalis*, as well as three species of two supposed new genera from *Bathygadus* spp., *Gadomus arcuatus*, and *V. mucocephalus*, all from the Gulf of Mexico/Caribbean region. Further species also

Received December 21, 1993. Accepted December 15, 1994.
J12518

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exist in unworked collections at The Natural History Museum, London, and elsewhere.

This work describes a new species of the opecoelid genus *Allopodocotyle* Pritchard, 1966, a genus not previously reported from deep-sea fishes, from *Coryphaenoides* (*Chalinura*) *mediterraneus* from the NE Atlantic region. This is one of two plagioporine opecoelids in the Collection of The Natural History Museum, London, from this host. *Coryphaenoides* (*C.*) *mediterraneus* is a benthopelagic fish occurring at a depth of 1200–3000 m on the Atlantic continental slope between 30 and 60°N and off the Azores, and in the western Mediterranean at 2300–2700 m; it feeds on small benthic invertebrates (Geistdoerfer 1986).

Materials and methods

The specimens were collected by Dr. R.A. Bray and Ms. M. Spencer Jones aboard the NERC RRS "Challenger" during two cruises in 1991. They were fixed live in Berland's fluid and stored in 80% alcohol. Whole mounts were stained in Mayer's paracarmine and mounted in Canada balsam. Serial sections were stained with Mayer's haematoxylin and eosin, and mounted in thin Canada balsam. The measurements given below are in micrometres, with the mean in parentheses.

Results

***Allopodocotyle margolisi* n.sp.** Figs. 1–3

Type host: *Coryphaenoides* (*Chalinura*) *mediterraneus* (Giglioli, 1893).

Site: Intestine.

Type locality: N. Rockall Trough, N. Atlantic Ocean off western Scotland (56°44′N, 09°44.5′W, depth 1745–1760 m; collected 19.11.1991 by RAB).

Other localities: N. Rockall Trough, (57°18′N, 10°24′W, depth 2220 m; collected 19.11.1991 by RAB); (57°10.2′N, 10°22.8′W, depth 2195 m; collected 17.2.1991 by MSJ).

Type material: The Natural History Museum, London, BM(NH) Reg. Nos: holotype 1992.3.24.33; paratypes 1992.3.24.34-42, 1992.7.1.186-188, 1992.7.28.28-31.

Description (based on 19 whole-mounted specimens, 10 of which were measured and two sets of serial sections)

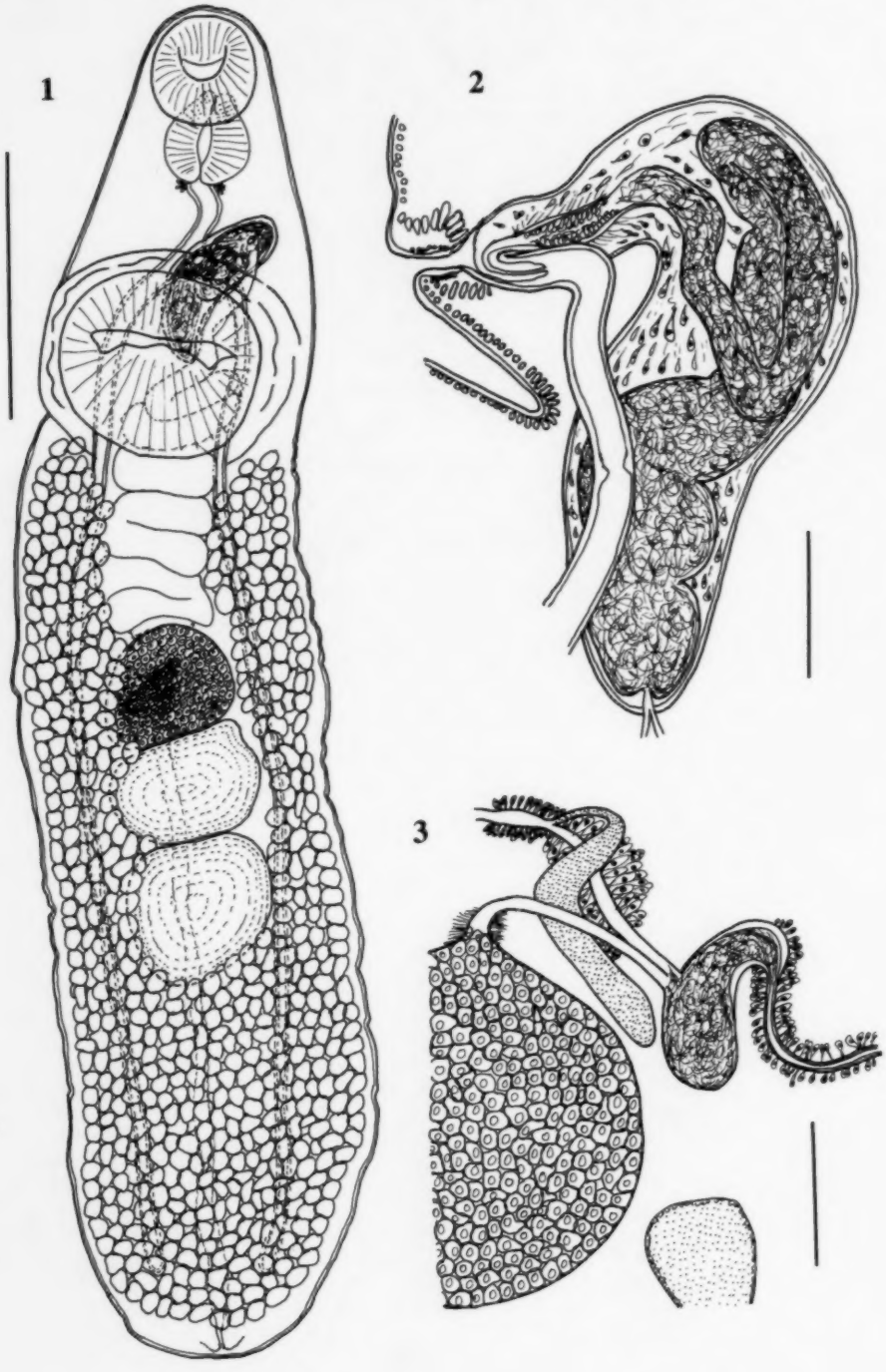
Body somewhat dorso-ventrally flattened, elongate-fusiform, with forebody more tapered than hindbody which has parallel sides; length 1440–3640 (2670); maximum breadth 350–920 (640); widest point at level of ventral sucker or throughout most of hindbody. Forebody 360–820 (590); 19–25 (22)% of body length. Tegument unarmed. Oral sucker simple; ventro-terminal; relatively small, 150–275 × 131–252 (217 × 197); with muscular thickening on posterior wall, but no distinct post-oral ring. Ventral sucker transversely oval; 232–440 × 266–475 (314 × 367); embedded on ventral eminence that forms anterior and lateral fold, but with no distinct, muscular circum-acetabular fold. Sucker-ratio 1:1.69–2.06 (1:1.86).

Prepharynx short; muscular; surrounded by layer of small gland-cells. Pharynx large, 101–204 × 90–202 (172 × 145); powerful; broad, often subpyriform, narrowing slightly anteriorly. Oesophagus relatively long, 75–225 (164); broad; surrounded anteriorly by layer of gland-cells. Intestinal bifurcation just anterior to or at level of anterior margin of ventral sucker. Caeca long, straight, relatively narrow; passing posteriorly in dorso-lateral plane; ending blindly close to posterior extremity.

Testes 2; contiguously tandem; inter-caecal in mid-hindbody; entire but somewhat variable in shape which may be affected by compression caused by their proximity; anterior 157–297 × 191–455 (241 × 302), often transversely oval; posterior 194–408 × 198–466 (299 × 306), sometimes longitudinally oval. Vasa efferentia leave testes antero-dorsally, pass forward dorsally to ovary and uterine field, and unite at base of cirrus-sac. Cirrus-sac medium-sized, length 146–455; relatively thin walled; broad; extends from region just anterior to genital pore obliquely to end in middle of body dorsally to anterior half (very occasionally posterior half) of ventral sucker; posterior extremity 58–204 (147) posterior to anterior margin of ventral sucker; large proximal region broad, sausage-shaped, tapers proximally and may curve around behind genital pore. Seminal vesicle internal, tubular, broad and straight or sinuous (occasionally looped) proximally and narrow and often coiled or looped distally. Pars prostatica short; tubular; in distal region of cirrus-sac; lined by typical "anuclear blebs"; external gland-cells surround pars prostatica and parts of seminal vesicle and ejaculatory duct. Ejaculatory duct forms short cirrus that may loop back into metraterm. Genital atrium small. Genital pore sinistrally ventro-sublateral at about mid-oesophageal level; often present on slight eminence and surrounded by muscular thickening of body-wall that appears to form sphincter.

Ovary entire, sub-oval to sub-pyriform (inverted heart shaped in one immature specimen, sub-campaniform in one mature specimen); 79–310 × 105–373 (221 × 245), usually smaller than testes (larger in one specimen with no eggs); immediately and contiguously pre-testicular. Oviduct leaves ovary antero-dorsally, loops dorsally or postero-dorsally, unites briefly with antero-ventral region of seminal receptacle as it reflexes anteriorly and enters Mehlis' gland. Mehlis' gland cells of two types; long region of large, indistinct cells proximally; short region of small deeply staining cells distally. Duct from vitelline reservoir appears to unite with oviduct approximately between two regions of Mehlis' gland. Seminal receptacle flask shaped; small, dorsal to ovary. Laurer's canal present; muscular; leaves muscular, tapered neck of seminal receptacle antero-dorsally and opens dorsally to left caecum at level antero-lateral to ovary; surrounded throughout its length by gland-cells. Uterus coiled inter-caecally between ovary and ventral sucker; terminal limb passes dorsally to ventral sucker to left of cirrus-sac. Metraterm short; muscular; ventral to distal part of cirrus-sac; junction with rest of uterus may be surrounded by sphincter. Eggs operculate; thin shelled; collapsed in both whole mounts and serial sections; medium sized, 56–68 × 24–35 (59.5 × 30) [usually 58–60 × 28–32], with shorter eggs tending to be wider than longer ones as a result of collapse. Vitellarium follicular; lateral fields of small, tightly packed follicles extend full length of hindbody, confluent

Figs. 1–3. *Allopodocotyle margolisi* n.sp. Fig. 1. Entire worm, ventral view. Fig. 2. Terminal genitalia, lateral view, reconstructed from serial sections. Fig. 3. Ovarian complex, lateral view, reconstructed from serial sections; stippled regions are the vitelline reservoir and the anterior extremity of the excretory vesicle. *Scale bars*: Fig. 1, 500 μm; Figs. 2 and 3, 100 μm.



only in post-testicular zone; anterior limit varies between just posterior to ventral sucker and middle of ventral sucker, usually close to posterior margin of sucker; fields lie dorsally (usually), ventrally (always), laterally (always), and medially (always in post-testicular zone, occasionally elsewhere) to caeca. Vitelline reservoir small; antero-dorsal to ovary.

Excretory pore dorsally subterminal. Excretory vesicle I-shaped, tubular; reaches forward dorsally to testes and ends dorsally to ovary.

Discussion

The genus *Allopodocotyle* was erected by Pritchard (1966) for *Podocotyle*-like species with an entire, rather than lobed (normally tri-lobed) ovary. Yamaguti (1971) did not recognize this genus, considering it a subgenus of *Podocotyle* Dujardin, 1845; but it has been generally recognized subsequently, for example by Bray (1987) and Bartoli et al. (1989), who dealt with it in some detail. In relation to the major diagnostic characters, the present species fits well into this genus as defined by Bartoli et al. (1989). *Allopodocotyle margolisi* n.sp. is the only species of this genus to be recorded in the published literature from deep-sea fishes. Ignoring nominal species of *Allopodocotyle* from freshwater fishes, which may eventually need to be distinguished at the generic level on the basis of their short excretory vesicle, the other nominal species are mainly parasites of perciforms, such as sparids, in shelf waters. These shallow-water species were split by Bray (1987) into four groups, one of which contained species of questionable generic position from fishes off India and Pakistan. No new species of *Allopodocotyle* appear to have been described subsequent to Bray's work. The present material fits none of the other three groups, but it is most similar to Bray's group C that has testes in tandem rather than oblique. The group includes the following: *A. argyropsi* Madhavi, 1975; *A. atzi* (Nigrelli, 1940); *A. israelense* (Fischthal, 1980); *A. jaffensis* (Fischthal, 1980); *A. lutjanusi* Gupta and Ahmad, 1977; *A. mecoptera* (Manter, 1940); and *A. pedicellata* (Stossich, 1887). It differs from the members of this group, as outlined by Bray, in that the cirrus-sac does not extend into the hindbody. However, as shown by Bartoli et al. (1989), the cirrus-sac of one member of this group, *A. pedicellata*, a parasite of sparids in the Mediterranean region, in fact rarely extends into the hindbody. Nevertheless, *A. margolisi* n.sp. can be distinguished from this species by its greater sucker ratio (1:1.69–2.06 *vs.* 1:1.01–1.63), longer oesophagus and more anterior extent of the vitelline fields, which do not reach the ventral sucker in *A. pedicellata*.

I have considered the unnamed opecoelids described by Armstrong (1974) from bathyal fishes because, although unpublished, this is a major taxonomic work on the parasites of bathyal fishes. *Allopodocotyle margolisi* n.sp. can be distinguished from (*i*) "*Podocotyle* (*Podocotyle*) sp.n. #1, #2, and #3" from *Ventrifossa atlantica*, *V. atlantica*, and *Sphagemacrurus grenadae*, respectively, in egg size, length of the excretory vesicle, the size of the pharynx, and (or) the posterior extent of the uterus; (*ii*) "*Podocotyle* (*Neopodocotyle*) sp.n." from *Nezumia aequalis* in lacking a four-lobed ovary and uterine coils posterior to the ovary, and in the size of the eggs and pharynx; (*iii*) "Genus novum B sp. #1 and #2" from *Bathygadus* spp. and *Gadomus arcuatus* in its much smaller size, more antero-lateral position of the genital pore and more anterior extent of the lateral vitelline fields; and (*iv*) "Genus novum C species nova" from *V. mucocephalum* in that its genital pore is not dextrally submedian.

In view of the above comments, I consider the present material from *Coryphaenoides* (*Chalinura*) *mediterraneus* as a species new to science and name it *Allopodocotyle margolisi* n.sp. for Dr. Leo Margolis of the Pacific Biological Station, Nanaimo, Canada, in recognition of his major contributions to fish parasitology.

One interesting feature of this particular species is the tendency for the cirrus to penetrate the metraterm. One might have speculated that in the deep-sea parasitological encounters may be so infrequent that worms often reside singly in their hosts and thus self-insemination might be advantageous; but, although true for bathypelagic fishes, this is not apparently the case for those in benthopelagic waters that have a relatively high prevalence of digeneans (Campbell 1990). Furthermore, although opecoeline opecoelids with atrophied terminal genitalia and a uterine seminal receptacle would appear to be adapted for such a life style, plagioporines with a temporary or permanent intromittent organ and a canalicular seminal receptacle do not. It has been assumed (Gibson and Bray 1979) that, in forms with a canalicular seminal receptacle, Laurer's canal acts as a vagina rather than as a sperm-drain. If this is the case then the location of the cirrus of this species in the metraterm is puzzling.

Acknowledgements

Thanks are due to Dr. R.A. Bray and Ms. Mary Spencer Jones, who collected the material, the crew of the NERC RRS *Challenger*, Mr. D.W. Cooper for preparing serial sections, and Dr. R.A. Bray for commenting on the manuscript.

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A new digenean, *Didymocystis margolisi* n.sp. (Didymozoidae: Didymozoinae), from the skipjack tuna, *Katsuwonus pelamis*, of Japan

Shunya Kamegai and Jun Araki

Abstract: ***Didymocystis margolisi*** n.sp. (Digenea: Didymozoidae: Didymozoinae) is described from a marine fish, *Katsuwonus pelamis*, caught off the Pacific coast of central Japan. Worms were found in pairs in a round somewhat flattened cyst in the dorsolateral subcutaneous tissue of the host. The new species is characterized by the intestine being distended with dark ingesta from the host blood, the forebody attached near to central part of hindbody, elongated testes, and a highly branched ovary. Taxonomic problems of the genus *Didymocystis* and its allied genera are discussed. ***Didymocystis komoroensis*** (Nikolaeva and Dubina, 1978) n.comb. (Syn. ***Didymocystoides komoroensis***) is proposed and the genera *Coeliodidymocystoides* Yamaguti, 1970 and *Univitellodidymocystis* Yamaguti, 1970 are considered valid.

Résumé : Le présent article fournit une description de ***Didymocystis margolisi*** n.sp. (Digenea : Didymozoidae : Didymozoinae), isolé sur *Katsuwonus pelamis*, poisson capturé au large de la côte du Pacifique du centre du Japon. Les vers ont été trouvés disposés par paires dans un kyste arrondi plus ou moins déprimé dans le tissu sous-cutané dorsolatéral de l'hôte. Cette nouvelle espèce se caractérise par un intestin distendu par le sang de l'hôte, la partie antérieure du corps attachée près de la partie centrale de la partie postérieure du corps, des testicules étendus et un ovaire fortement ramifié. Les problèmes taxinomiques soulevés par le genre *Didymocystis* et les genres apparentés sont examinés. La désignation ***Didymocystis komoroensis*** (Nikolaeva et Dubina, 1978), n.comb. (Syn. ***Didymocystoides komoroensis***) est proposée et les genres *Coeliodidymocystoides* Yamaguti, 1970 et *Univitellodidymocystis* Yamaguti, 1970 sontconsidérés comme valides.
[Traduit par la Rédaction]

Introduction

Several yellow, flattened cysts were obtained from the dorsolateral subcutaneous tissue of a skinned raw skipjack tuna, *Katsuwonus pelamis*, caught off the Pacific coast of central Japan in July 1990 and purchased at a fish market in Tokyo. The cysts contained paired live didymozoids that are described herein as a new species.

Materials and methods

Worms were excysted with a needle and fine drawing brush, fixed with alcohol – formalin – acetic acid (AFA) under a cover glass with slight pressure, stained with Delafield's hematoxylin, and mounted in Canada balsam. The flukes were drawn with the aid of a camera lucida. Measurements are given in millimetres unless otherwise stated.

Received March 7, 1994. Accepted December 15, 1994.
J12520

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Didymocystis margolisi n.sp. Figs. 1 and 2

Host: *Katsuwonus pelamis* (skipjack tuna).

Location: Encysted in pairs in dorsolateral subcutaneous tissue.

Locality: Off the Pacific coast of central Japan.

Date: 9 July 1990.

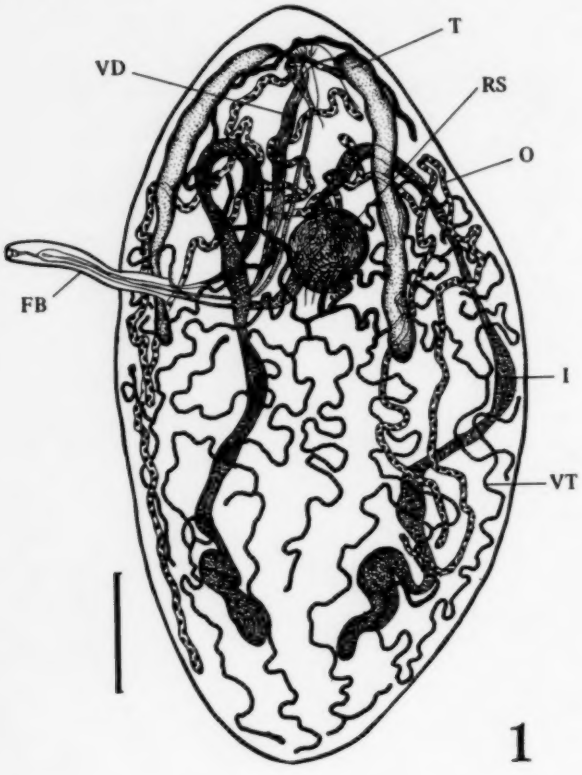
Holotype and paratypes: Deposited in the collection of the Meguro Parasitological Museum as Coll. No. 19599.

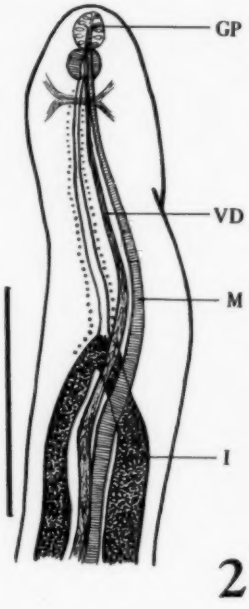
Etymology: The species is named in honor of Dr. Leo Margolis, Pacific Biological Station, Nanaimo, Canada.

Description (based on eight mature specimens)

Two hermaphroditic worms in each cyst are almost of the same size and shape and in contact with each other by the flattened side of the hindbody, with the forebodies between. Forebody slender, 1.400–2.500 long, with almost uniform breadth of 0.250–0.300, attached near to central part of ventral side of hindbody. Oral sucker terminal, cellular, 0.045–0.050 long by 0.052–0.068 wide. Pharynx muscular, 0.045–0.058 long by 0.052–0.068 wide. Esophagus slender, 0.400–0.560 long, surrounded by small glandular cells throughout its length, bifurcating 0.500–0.700 distant from anterior end of forebody. Intestinal ceca distended with dark, granular ingesta from host blood throughout their

Figs. 1 and 2. *Didymocystis margolisi* n.sp. Fig. 1. Entire worm, holotype, ventrolateral view. Scale bar = 1 mm. Fig. 2. Forebody, ventral view. Scale bar = 0.4 mm. FB, forebody; GP, genital pore; I, Intestine; M, metraterm; O, ovary; RS, receptaculum seminis; T, testis; VD, vas deferens; VT, vitellarium.





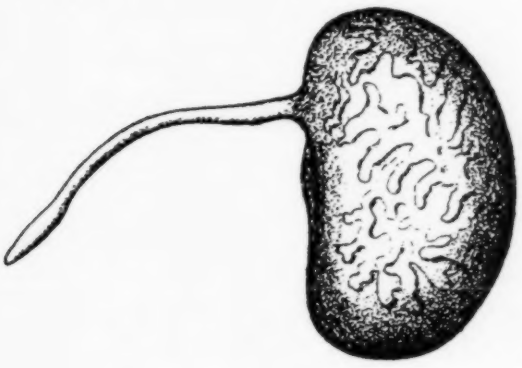
courses; narrow and straight in forebody, but, in hindbody, tortuous of zigzaged, first proceeding toward anterior end of hindbody, then turning back near anterior end to terminate blindly near posterior end of hindbody. Acetabulum absent.

Testes elongated, curved, 2.500–2.700 long by 0.100–0.270 wide, situated symmetrically and laterally in anterior half of hindbody; vasa efferentia joining to form a vas deferens 0.430 distant from anterior extremity of hindbody in holotype. Vas deferens tubular, 0.100 wide at its base, running forward along metraterm in median field of forebody. Common genital pore midventral to oral sucker. Ovary tubular, winding; stem running forward from genital junction, divided into right and left branches; right branch soon divided again into four long terminal branches, two of which extend to anterior extremity of hindbody, and two others which extend close to each other on right side of hindbody as far as posterior end of right intestinal cecum; left branch divided again into one short and two long terminal branches on left side of hindbody along with left intestinal cecum. Total number of terminal ovarian branches is 8 in holotype and 9 in one paratype. Receptaculum seminis rounded, large, 0.450–0.800 in diameter, median, ventral to genital junction, 1.820 distant from anterior extremity of hindbody in holotype. Vitellarium digitate, winding, divided posterodorsally to receptaculum seminis into five main branches, each of which is divided dichotomously two to three times into 18–29 terminal branches. Vitelline reservoir absent. Uterine coils first winding irregularly in peripheral area, turning backward along with vitelline branches, then forward at posterior extremity of hindbody, and again winding end to end several times, occupying all available space of hindbody, finally forming an elliptical, small median, egg reservoir. Metraterm well differentiated, muscular, running along with vas deferens in anterior part of hindbody and then proceedings into forebody. Eggs elliptical to bean shaped, 12–18 × 7–10 μm. Excretory vesicle not detected.

Discussion

This new species differs from all previously known species in the genus *Didymocystis* Ariola, 1902 in having the intestine filled with dark ingesta from the host blood, the origin of the forebody from near the middle of the hindbody, elongated testes and a highly branched ovary. Among previously named didymozoids, *Melanocystis kawakawa* Yamaguti, 1970 is the only species to contain dark ingesta of the host blood in the intestine (Yamaguti 1970). The genus *Melanocystis*, however, differs fundamentally from *Didymocystis* by having a transversely elongated hindbody.

Fig. 3. *Didymocystis reniformis* Ariola, 1902, after Ariola (1902). **Fig. 4.** *Didymosulcus katsuwonicola* (Okada, 1926) Pozdnyakov, 1990, after Ishii (1935).



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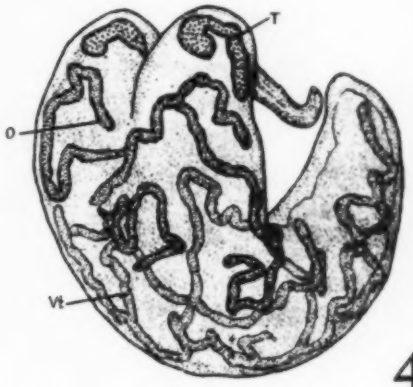

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Ariola (1902) erected the genus *Didymocystis* with *D. reniformis* Ariola, 1902 as the type species. Ishii (1935) synonymized *D. reniformis* with *Didymozoon thynni* Taschenberg, 1879 and accordingly designated *Didymozoon thynni* as the type species of *Didymocystis*. Pozdnyakov (1990) regarded *Didymozoon thynni* as a *species inquirenda* and retained *D. reniformis* as the type species. Yamaguti (1970) proposed a new genus, *Didymocystoides*, with *Didymocystoides bifasciatus* Yamaguti, 1970 as the type species. We agree with Pozdnyakov (1990) that *Didymocystoides* is a synonym of *Didymocystis* because both have a reniform hindbody with a smooth ventral surface (Fig. 3) (cf. Ariola 1902; Yamaguti 1970, 1971). Pozdnyakov (1990) created a new genus, *Didymosulcus*, with *D. katsuwonicola* (Okada, 1926) as the type species, for those species possessing a hindbody that is longitudinally bilobed in the ventral surface (Fig. 4), and included 16 other species in this genus as well. *Didymocystoides komoroensis* Nikolaeva and Dubina, 1978, which Pozdnyakov (1990) did not mention, should be transferred to *Didymocystis* as *D. komoroensis* (Nikolaeva and Dubina, 1978) n.comb. because of the unlobed reniform hindbody (Nikolaeva and Dubina 1978).

Pozdnyakov (1990) also synonymized *Coeliodidymocystoides* Yamaguti, 1970 and *Univitellodidymocystis* Yamaguti, 1970 with *Didymocystis*. However, the hindbody in *Coeliodidymocystoides* is not reniform but dorsoventrally flattened and uniformly rounded (Yamaguti 1970); and *Univitellodidymocystis* has a single vitellarium (Yamaguti 1970), which is one of the most important features distinguishing it from allied genera with branched vitellaria. Consequently, we consider these two genera valid.

Including the present new species, the total number of species contained within the genus *Didymocystis* is 19. They are as follows: *D. reniformis* Ariola, 1902; *D. scomberomori* (MacCallum and MacCallum, 1916); *D. semiglobularis* Ishii, 1935; *D. acanthocybii* Yamaguti, 1938; *D. alalongae* Yamaguti, 1938; *D. opercularis* Yamaguti, 1938; *D. submentalis* Yamaguti, 1938; *D. singularis* (Job, 1966); *D. bifasciatus* (Yamaguti, 1970); *D. buccalis* (Yamaguti, 1970); *D. exiguus* (Yamaguti, 1970); *D. intestinomuscularis* (Yamaguti, 1970); *D. oesophagicola* (Yamaguti, 1970); *D. pectoralis* (Yamaguti, 1970); *D. pinnicola* (Yamaguti, 1970); *D. sphyraenae* (Hussain, Hanumantharao and Shyamasundari, 1985); *D. visakhapatnamensis* (Hussain, Hanumantharao and Shyamasundari, 1985); *D. komoroensis* (Nikolaeva and Dubina, 1978) n.comb.; and *D. margolisi* n.sp.

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A new species of Megalophallus (Digenea: Microphallidae) from the clapper rail, other birds, and the littoral isopod *Ligia baudiniana*

Robin M. Overstreet and Richard W. Heard

Abstract: ***Megalophallus reamesi*** n.sp. infected the clapper rail, ruddy turnstone, black-bellied plover, and the isopod *Ligia baudiniana*. This precociously developed digenean occurred in the mid-Florida Keys, Florida, and in Pine Cay, Turks and Caicos, British West Indies, but not in numerous other examined localities from New York to Texas. It is most similar in body size (1.0–1.6 vs 1.0–1.1 mm) and structure of its copulatory organ to *M. diodontis* Siddiqi and Cable, 1960, but its copulatory organ has 23–39 micropapillae rather than 12–19, the species has rudimentary intestinal ceca rather than short fully developed ones, and an isopod rather than a portunid crab serves as the second intermediate host. The encysted metacercaria, occurring in the hemocoel of the isopod, was typically encapsulated with host connective tissue incorporating several host chromatophores.

Résumé : Le ***Megalophallus reamesi*** n.sp. infecte le râle gris, le tournepierre à collier, le pluvier argenté et l'isopode *Ligia baudiniana*. Ce digénien à développement précoce a été retrouvé dans la région centrale des Keys, en Floride, et à Pine Cay (Turks et Caicos), dans les Antilles britanniques, mais non dans de nombreuses autres localités étudiées, situées de New York au Texas. Par sa taille (1,0–1,6 c. 1,0–1.1 mm) et la structure de son organe copulateur, il ressemble beaucoup au *M. diodontis* Siddiqi et Cable, 1960, mais son organe copulateur compte 23–39 micropapilles contre 12–19, l'espèce possède des caecums intestinaux rudimentaires et non des caecums courts mais entièrement développés, et elle a un isopode au lieu d'un crabe portunidé comme deuxième hôte intermédiaire. Les métacercaires enkystées, présentes dans l'hémocèle de l'isopode, étaient typiquement encapsulées dans le tissu conjonctif avec plusieurs chromatophores de l'hôte.

[Traduit par la Rédaction]

A new species of *Megalophallus* Cable, Connor, and Balling, 1960 (Digenea: Microphallidae) was collected from the clapper rail, *Rallus longirostris* Boddaert, 1783, and other birds in insular areas in the mid-Florida Keys. This report describes this species, previously reported as "*Megalophallus* sp. Heard" by Prévot and Deblock (1970), Deblock (1971), and Prévot (1972), as well as provides data on infections in its second intermediate host, the isopod *Ligia baudiniana* H. Milne Edwards, 1840, in the Florida Keys and British West Indies.

Materials and methods

Specimens of the clapper rail and other birds were collected and examined for parasites in Florida during 1966, 1968, and 1970 in the manner previously reported by Heard (1970) when he examined them from other geographic locations. Various isopods were collected for general parasitological examination from Florida in 1966, 1968, and 1991 as well as from numerous coastal localities in the Caribbean Sea, Atlantic Ocean, and Gulf of Mexico from 1966 to 1993. Infections of the new species, however, were restricted to the Florida Keys and British West Indies.

Living specimens of the new species were examined using light microscopy to determine details of the excretory and reproductive systems. Some specimens of metacercariae were cultured in saline at 35–40°C for various periods up to 3 days to obtain reared egg-bearing specimens. Individuals were heat killed in hot saline, either under slight coverslip pressure or without pressure, and immediately fixed in AFA (ethyl alcohol, formalin, and acetic acid). Most specimens were then stained with Harris' hematoxylin or Van Cleave's hematoxylin and mounted in Canada balsam using standard procedures. Two were post-fixed in OsO_4, rinsed in 0.1 M cacodylate, dehydrated through an alcohol series, dried in a critical-point dryer, sputter-coated with gold, and examined with a Jeol JSM-T330 scanning electron microscope. Others were embedded in paraffin, sectioned at 5 μm, and stained with Harris' hematoxylin and eosin. Line drawings were prepared with the aid of a

Received March 14, 1994. Accepted December 7, 1994.
J12675

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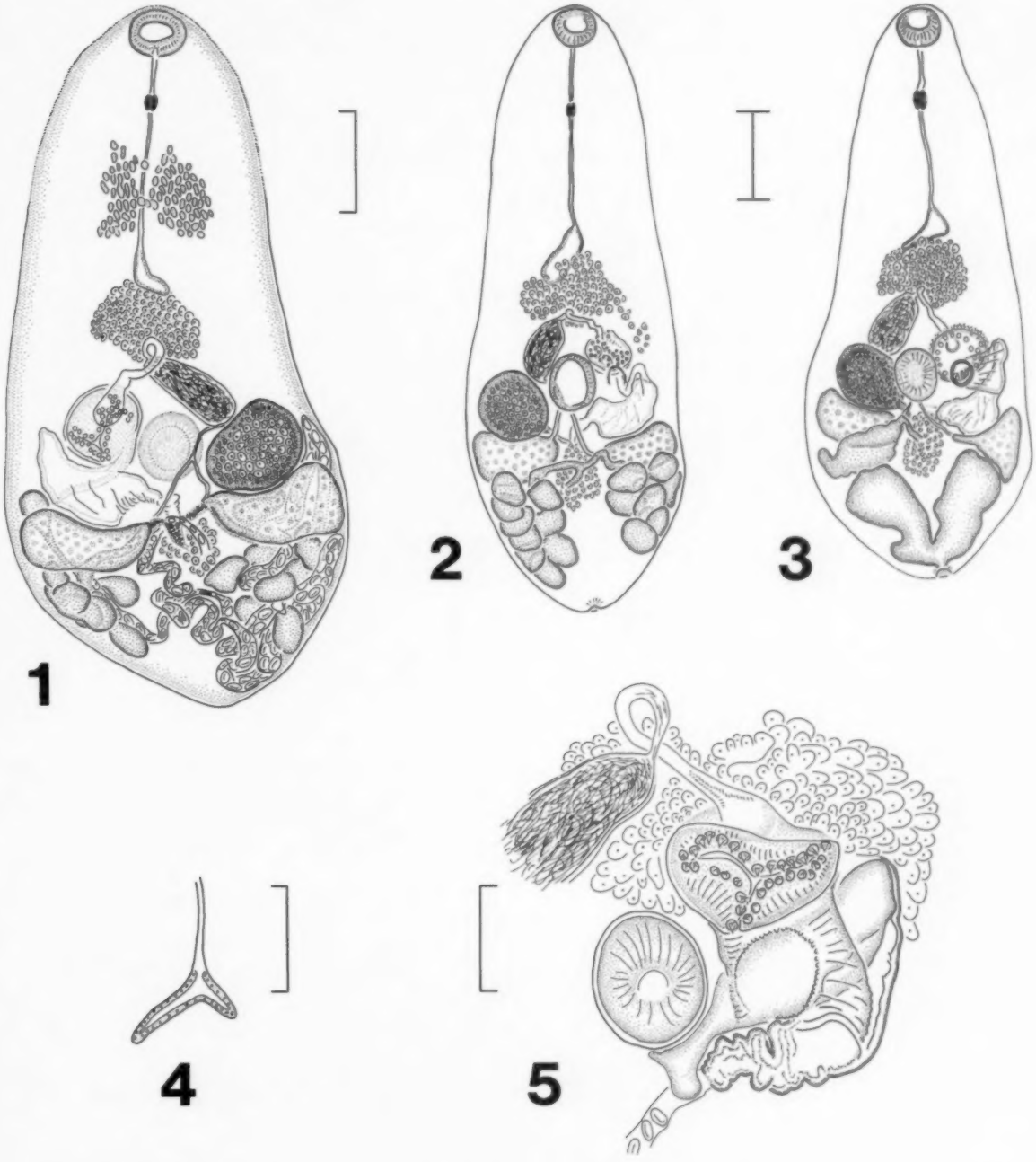
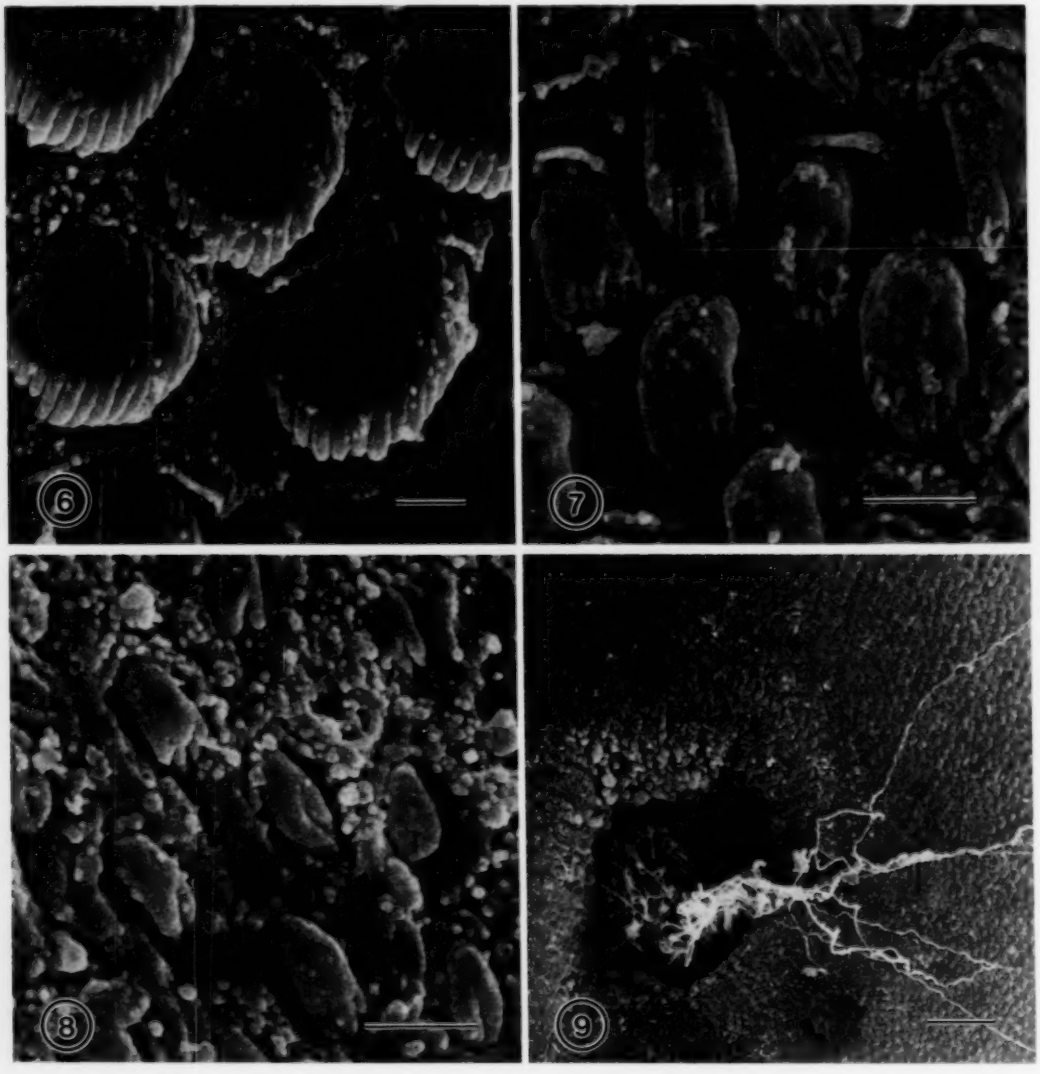
Figs. 1–5. ***Megalophallus reamesi*** **n.sp. Fig. 1. Whole mount of holotype from clapper rail on Molasses Keys, Florida, to show uterus containing relatively few eggs; under slight pressure, dorsal view, scale bar = 200 μm. Fig. 2. Specimen of metacercaria from isopod *Ligia baudiniana* at Pine Cay of Turks and Caicos, British West Indies; note vitelline follicles and large gonads, ventral view. Fig. 3. Metacercaria from isopod on Molasses Keys showing fused vitelline follicles, large gonads, and conspicuous excretory vesicle; ventral view, scale bar = 200 μm and pertains to both Figs. 2–3. Fig. 4. Bifid ceca in metacercaria from clapper rail, scale bar = 100 μm. Fig. 5. Terminal genitalia of adult specimen from clapper rail in Molasses Keys, ventral view, scale bar = 100 μm.**



Figs. 6–9. Scanning electron micrographs of *Megalophallus reamesi* n.sp. Figs. 6–8. Scale-like tegumental spines. Fig. 6. At pharyngeal level, scale bar = 1 μm. Fig. 7. At a level just posterior to pharynx, scale bar = 1 μm. Fig. 8. Near posterior end of body, scale bar = 1 μm. Fig. 9. Genital pore with extruded spermatozoa, scale bar = 10 μm.



drawing tube. Measurements are given in micrometres unless stated otherwise.

Results

Megalophallus reamesi n.sp. Figs. 1-15

Synonyms

Megalophallus sp. Heard of Prévot and Deblock, 1970
Megalophallus sp. Heard of Deblock, 1971
Megalophallus sp. Heard of Prévot, 1972.

Diagnosis: Body 1.0–1.6 mm long; intestinal ceca rudimentary; male copulatory organ with 23–39 micropapillae; prostatic cells extensive in number between ceca and level of seminal vesicle.

Description (based on 17 specimens; 10 from the clapper rail, with 1 having only few immature eggs, and 7 excysted metacercariae from the isopod, with three of those containing numerous eggs because worms were cultured in saline; measurements in parentheses refer to holotype): Microphallidae, Microphallinae. Body pyriform, 988–1587 (1375) long by 458–729 (654) in maximum width (at or near testicular level). Tegument spinose, with scale-like spines most abundant and largest (6) near anterior end (at mid-esophageal level) where distal margin of scales consisting of several finger-like projections (Fig. 6); scales

diminish in size and number posteriorly to posterior end, with distal margins consisting of fewer projections (Figs. 7–8). Oral sucker subterminal, opening ventrally, 70–103 (91) long by 86–120 (111) wide. Acetabulum embedded, 92–179 (130) long by 86–162 (125) wide. Sucker width ratio 1:1.0–1.7 (1:1.1). Forebody 48–62% (57%) of body length. Pharynx 22–43 (43) long by 11–23 (19) wide. Prepharynx 56–131 (78) long, 1.5–6.1 (1.8) times longer than length of pharynx. Esophagus 99–320 (270) long, 2–5 (3.5) times longer than prepharynx. Intestinal ceca rudimentary but with epithelial lining, sac-like to weakly bifurcate (Figs. 1–4), with one cecum longer than other, from nondetectible to 112 (79) in length.

Testes irregular in shape, symmetrical, laterally elongate, postacetabular; right testis 69–161 (161) long by 124–268 (201) wide; left testis 74–118 (118) long by 114–255 (255) wide. Posttesticular space 21–31% (25%) of body length. Seminal vesicle elongate, usually slightly arcuate, 126–223 (196) long by 43–117 (78) wide, lying diagonally anteriodextral to acetabulum, not overlapping it dorsally. Vas efferentia extending from or near medial margin of testes to acetabular level, merging to form vas deferens; vas deferens with length approximately that of acetabulum, entering seminal vesicle at posterior margin. Pars prostatica tubular, looped, with distally associated ejaculatory duct opening into base of copulatory organ, associated with numerous prostatic cells; prostatic cells bunched together in mass usually located between ceca and seminal vesicle, not enclosed in membrane. Male copulatory organ entire, usually larger but occasionally about equal in size to acetabulum, subspherical or irregular in shape depending on state when fixed, anterio-sinistral to acetabulum, bearing 23–39 (39) micropapillae; micropapilla shaped like "upholsterer's tack" (Fig. 5). Genital atrium thin walled, sinistral to acetabulum and often extending posterior to it (Fig. 5); genital pore usually oval, smaller than acetabular outline but typically larger than acetabular depression, with contractile margin, sinistral to and separate from acetabulum.

Ovary smooth, dextral at level of middle to posterior margin of acetabulum, anterior to or contiguous with right testis, usually longer than wide, 92–267 (267) long by 60–195 (147) wide. Seminal receptacle absent. Ootype with associated Laurer's canal and Mehlis' gland lying medially intertesticular or at a level just posterior to testes; Laurer's canal opening dorsally at level of conspicuous Mehlis' gland. Vitellaria in lateral groups, with 1–11 (7) vitelline follicles on left and 1–13 (10) vitelline follicles on right when evident, more poorly defined in gravid specimens and sometimes appearing fused in metacercariae or adults with few eggs, ranging from testicular level to just beyond midlevel of posttesticular space; follicles measuring 15 long by 15 wide to 143 long by 35 wide. Uterus voluminous, occupying most available space in hindbody of heavily gravid specimens, with lateral loops extending up to level of male copulatory organ. Metraterm thick walled, sinistral, anterior to left testis, with conspicuous enfoldings, 177–294 (251) long by 80–162 (143) wide, joining genital atrium at its sinistral-most margin. Eggs 19–26 (19–21) long by 10–14 (11–13) wide when well developed, usually 20–23 by 11–14.

Excretory system with vesicle variable in shape from Y to V to U shaped; vesicle extending anteriorly to or near testes; pore subterminal; flame cell formula typical for microphallids, 2[(2 + 2) + (2 + 2)] = 16.

Encysted metacercaria: Cysts usually spherical or subspherical but occasionally ellipsoidal when lodged among midgut gland tubules; fresh cysts without host encapsulation 670–820 long by 550–660 wide; fixed cysts without host encapsulation 471–693 long by 370–530 wide. Cyst wall relatively thick, multilayered; internal layer extremely thin; mid-wall hyaline, two layered, totaling 23–31 thick with each layer usually equal in thickness (Figs. 11, 12, 14); outer layer thin, probably of host origin (Fig. 11); host connective tissue covering often incorporating host chromatophores, of varying thickness (Figs. 11–15).

Vertebrate and type host: *Rallus longirostris* Boddaert, 1783, clapper rail; Other vertebrate hosts: *Arenaria interpres* (Linnaeus, 1758), ruddy turnstone; *Pluvialis squatarola* (Linnaeus, 1758), black-bellied plover.

Site of Infection: ileum (lower half), ceca, and large intestine.

Second intermediate host: *Ligia baudiniana* H. Milne Edwards, 1840.

Site of infection: hemocoel, often attached to tubules of midgut gland.

Type locality: Molasses Keys (near Marathon), Monroe County, Florida, United States; other localities: Ohio Key and Hardup Key, Monroe County, Florida; Pine Cay of Turks and Caicos, British West Indies.

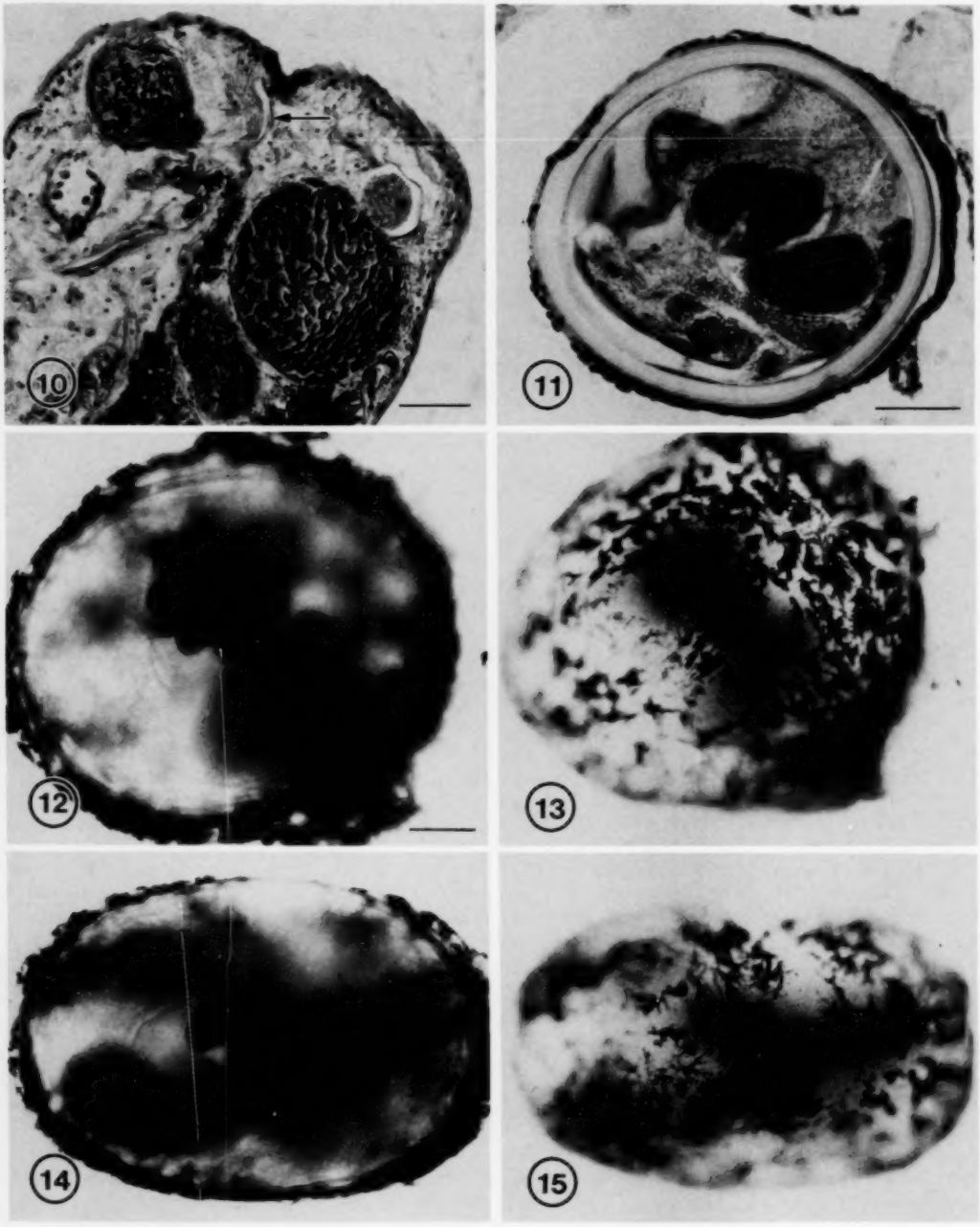
Deposition of specimens: Holotype, National Museum Parasite Collection, Beltsville, Md., USNM Helm. Coll. No. 84492; paratypes, USNM Helm. Coll. No. 84493 (one adult) and USNM Helm. Coll. No. 84494 (one excysted metacercaria); H.W. Manter Laboratory, University of Nebraska State Museum, Lincoln, Nebr., HWML No. 38102 (one adult) and HWML No. 38103 (one excysted metacercaria); and Gulf Coast Research Laboratory Museum, Ocean Springs, Miss., GCRLM No. 1330 (one adult).

Etymology: This species is named in honor of Robert C. Reames, a friend and good naturalist.

The new digenean occurred in the intestines of 9 of 13 specimens of the clapper rail (2–15 specimens per bird) examined in 1966 and 1968 from Molasses Keys, Hardup Key, and Ohio Key in the mid-Florida Keys. It did not occur in 146 other specimens examined at 11 locations from Louisiana to New York, excluding Florida (Heard 1970), nor in other clapper rails collected from mangrove habitats at Tampa Bay (10 birds) and Vero Beach (10 birds), Florida. Two specimens of both the ruddy turnstone and black-bellied plover were examined from the mid-Florida Keys in March 1968, and they had an average of 5 and >60 specimens, respectively.

With the exception of crustacean infections in *Ligia baudiniana* at Pine Cay, Turks and Caicos, British West Indies, we have seen the new species in *L. baudiniana* from the mid-Florida Keys only. We have examined specimens of this and other marine intertidal isopods such as *L. exotica* Roux, 1828 in Florida from other keys in the Florida Keys, Biscayne Bay, Vero Beach, Tampa Bay, and the panhandle as well as in Alabama, Mississippi, Louisiana,

Figs. 10–15. Light micrographs of *Megalophallus reamesi* n.sp. Fig. 10. Tangential histological section through ovary and both testes of adult showing Laurer's canal near dorsal surface (arrow), scale bar = 100 μm. Fig. 11. Histological section through area of vitelline follicles and uterus of encysted metacercaria showing clear layers of cyst wall and overlying host layers. Chromatophores are obvious in outer host encapsulation, scale bar = 100 μm. Figs. 12–15. Entire fixed metacercariae cleared in glycerine to show chromatophores in host encapsulation, scale bar = 100 μm. Fig. 12. Optical section through middle of cyst showing worm and cyst wall. Fig. 13. Optical section at top of same cyst shown in Fig. 12. Figs. 14–15. Same optical levels as in Figs. 12–13 but showing an elliptical rather than typically subspherical cyst configuration.



and Texas without observing an infection. In March 1968, 10 of 13 examined specimens of the isopod from Molasses Keys were infected with from 1 to 40 metacercariae, with an average of 8.8 cysts per infected host. In October 1991, 10 of 15 specimens 6–11 mm in length (not including antennae or telson) from the same location also had the infection. These had a range of only 1-6 metacercariae, with an average of 2.7 per infected host. In 1989 and 1990 at Pine Cay, Turks and Caicos, where *L. baudiniana* is common (Schotte et al. 1991), specimens exhibited light infections. Only one metacercaria was present in a group of 10 isopods examined each year. Even though not seen or examined, the presence of the clapper rail on Pine Cay was evident from its distinctive call, and other potential avian hosts were present.

Discussion

Based on having a copulatory organ with micropapillae, *Megalophallus reamesi* n.sp. is more closely related to M. pentadactylus Cable, Connor, and Balling, 1960 and *M. diodontis* Siddiqi and Cable, 1960 than to the few other species named in or transferred to the genus *Megalophallus*. It clearly differs from *M. pentadactylus* by being larger (1.0–1.6 mm long rather than 0.4–0.5 mm) and having many more than five micropapillae (Cable et al. 1960) on the copulatory organ. It is similar in size to *M. diodontis*, a species considered by Yamaguti (1971) as the type species of *Pseudomegalophallus* Yamaguti,1971, but has 23–39 micropapillae rather than 12-19 and has shorter, if any, cecal arms. *Megalophallus reamesi* has an isopod as the second intermediate host rather than a portunid crab like *M. diodontis* (see Siddiqi and Cable 1960).

We combined for the description of *M. reamesi* precociously developed specimens of metacercariae cultured in saline at 35–40°C for up to 3 days with those of adults because there was no difference in size of eggs or body. Eggs were produced in some specimens within 1 day of culture, and poorly developed eggs occasionally occurred in metacercariae in situ. Based on a series of paratype and other adults from rails, those with a uterus packed with eggs had a relatively small ovary, smaller and less basophilic testes, and few small indistinct vitelline follicles when compared with adults having relatively few eggs in the uterus or with metacercariae from isopods. Even though the smallest paratype was a metacercaria, some metacercariae from isopods were considerably larger than adults from birds. Specimens from the ruddy turnstone and black-bellied plover included worms gravid and without eggs that all exhibited characteristics similar to those of material from the clapper rail.

As individuals became progressively gravid, products from the ovary, testes, and vitelline follicles were apparently not restored like in many digeneans, and the organs gradually decreased in size. This apparent inability for *M. reamesi* to continuously produce eggs for a long period, its ease to produce eggs when cultured in saline at 35–40°C, and its rudimentary gut are all features indicating that this helminth is short lived in the bird host. Consequently, these features support an infection that can be restricted to small geographic areas, areas where the migrating bird host can acquire an infection and deposit the worm eggs in the vicinity of the snail host prior to the bird's departure. The snail hosting *M. reamesi* probably is confined to the American Mediterranean.

The metacercaria of *M. reamesi* occurs in the hemocoel of the isopod host loosely attached to the tubules of the midgut gland (also called hepatopancreas) or the hemocoel wall. As shown in Figs. 11–15, the typically spherical shape can be elongate, making identification based on shape confusing. The chromatophores surrounding most specimens made individuals easy to detect and easy to distinguish from most other microphallids. The wall of the metacercaria is thick relative to that of some species, such as *M. diodontis*. The reason for this may be that the clapper rail and other avian hosts of *M. reamesi* have a gizzard, and excysted worms from thin-walled, less resilient cysts of *M. diodontis* may not be able to survive passage through the gizzard to the lower intestine of these birds. *Megalophallus diodontis* infects fishes and mammals (Siddiqi et al. 1960; R.W. Heard, unpublished data).

The report of "*Megalophallus* sp. Heard" presented by Prévot and Deblock (1970) and repeated by Deblock (1971) and Prévot (1972) listing *Lygia* [sic] *exotica* from the Gulf of Mexico as the second intermediate host is misleading. The tentative identification of the isopod by Heard was in error; the parasite material used for those reports included the holotype and some of the paratypes that we describe herein. The location is also misleading, because the keys where the isopods had infections occurred in a transition area between the Gulf of Mexico (Hardup Key in Florida Bay) and the Atlantic Ocean (Molasses Keys off Hawk Channel).

Acknowledgments

We thank Robert C. Reames for his enthusiasm and assistance during the early phases of this study. Albert Bush of Brandon University, Manitoba, and Lidia Stuck, Ronnie Palmer, Susan Powell, and Jean Jovonovich-Alvillar of the Gulf Coast Research Laboratory provided technical assistance. William and Eileen Joyce of Key Colony Beach, Florida, provided lodging and use of their boat. This study was conducted with the cooperation of the U.S. Department of Agriculture via CSRS grant No 92-8808-6920 and the Oakleigh L. Thorne Foundation, through a grant to Stephen Spotte. It is contribution No. 10 of the Turks and Caicos Coral Reef Ecology Program.

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Geographical variations in abundance of larval anisakine nematodes in Atlantic cod (*Gadus morhua*) and American plaice (*Hippoglossoides platessoides*) from the Gulf of St. Lawrence

France Boily and David J. Marcogliese

Abstract: Atlantic cod (*Gadus morhua*) and American plaice (*Hippoglossoides platessoides*) collected from various sites in the Gulf of St. Lawrence (NAFO divisions 4RST) were examined for larval anisakine nematodes in 1990 and 1992. Sealworm larvae (*Pseudoterranova decipiens*) were more abundant in southern Gulf (4T) than in northern Gulf (4RS) cod and plaice. The heaviest sealworm infections occurred in fish from St. Georges Bay, Nova Scotia. Abundance of *P. decipiens* in cod from St. Georges Bay was significantly lower in 1992 than in 1990. *Anisakis simplex* and Contracaecinea (*Contracaecum*/*Phocascaris* spp.) were most abundant in cod from the northern Gulf. American plaice were not heavily infected with *A. simplex* or Contracaecinea. Geographical and temporal variations in abundance of larval anisakine nematodes in fish may reflect distribution and abundance of the phocid and cetacean definitive hosts and variations in water temperatures.

Résumé : Des morues franches (*Gadus morhua*) et des plies canadiennes (*Hippoglossoides platessoides*) ont été capturées à différents sites du golfe du Saint-Laurent (divisions 4RST de l'OPANO) en 1990 et 1992, et ont été examinées pour la présence de stades larvaires de nématodes anisakinés. Les larves de ver du phoque (*Pseudoterranova decipiens*) étaient plus abondantes dans les morues et les plies du sud du Golfe (4T) que dans celles du nord du Golfe (4RS). Les niveaux d'infection les plus élevés ont été rencontrés chez les poissons provenant de la Baie Saint-Georges, en Nouvelle-Écosse. Cependant, l'abondance du *P. decipiens* dans la morue de la Baie Saint-Georges a diminué de façon significative de 1990 à 1992. *Anisakis simplex* et les contracaecinés (*Contracaecum*/*Phocascaris* spp.) étaient plus abondants dans la morue du nord du Golfe. Les plies étaient peu infectées par les *A. simplex* et les contracaecinés. Les variations géographiques et temporelles d'abondance des larves de nématodes anisakinés dans les poissons semblent être liées à la distribution et à l'abondance des phoques et des cétacés, qui sont les hôtes finaux, et aux variations de températures de l'eau.

Introduction

Cod and many other marine fishes from eastern Canadian and European waters are commonly infected with anisakine nematodes such as sealworm (*Pseudoterranova decipiens* (Krabbe, 1878)), whaleworm (*Anisakis simplex* (Rudolphi, 1809)), and *Contracaecum* (*Phocascaris*) spp. Larval *P. decipiens* in fish fillets are a major cosmetic problem and affect the marketability of the product and the profitability of fish processing (Malouf 1986). Both *P. decipiens* and *A. simplex* can be pathogenic to humans when consumed in raw or undercooked seafood, although whaleworm occurs only occasionally in fish fillets and is not easily detected (Smith and Wootten 1978). Larval Contracaecinea (*Contracaecum*/*Phocascaris* spp.) are seldom a cosmetic or health concern, as they mostly occur in the body cavities of fish (McClelland et al. 1983*a*). The three anisakine taxa use invertebrates and fish as intermediate and paratenic hosts, and mature in the gastrointestinal tract of marine mammals (McClelland et al. 1983*a*).

Levels of larval sealworm infection in fillets of Atlantic cod (*Gadus morhua*), American plaice (*Hippoglossoides platessoides*), and other marine fishes from the Gulf of St. Lawrence were documented by Templeman et al. (1957), Scott and Martin (1957, 1959), and Wiles (1968). More recently, McClelland et al. (1983*a*, 1983*b*, 1985, 1987) examined the abundance of anisakine nematodes in cod

Received March 30, 1994. Accepted March 3, 1995.
J12799

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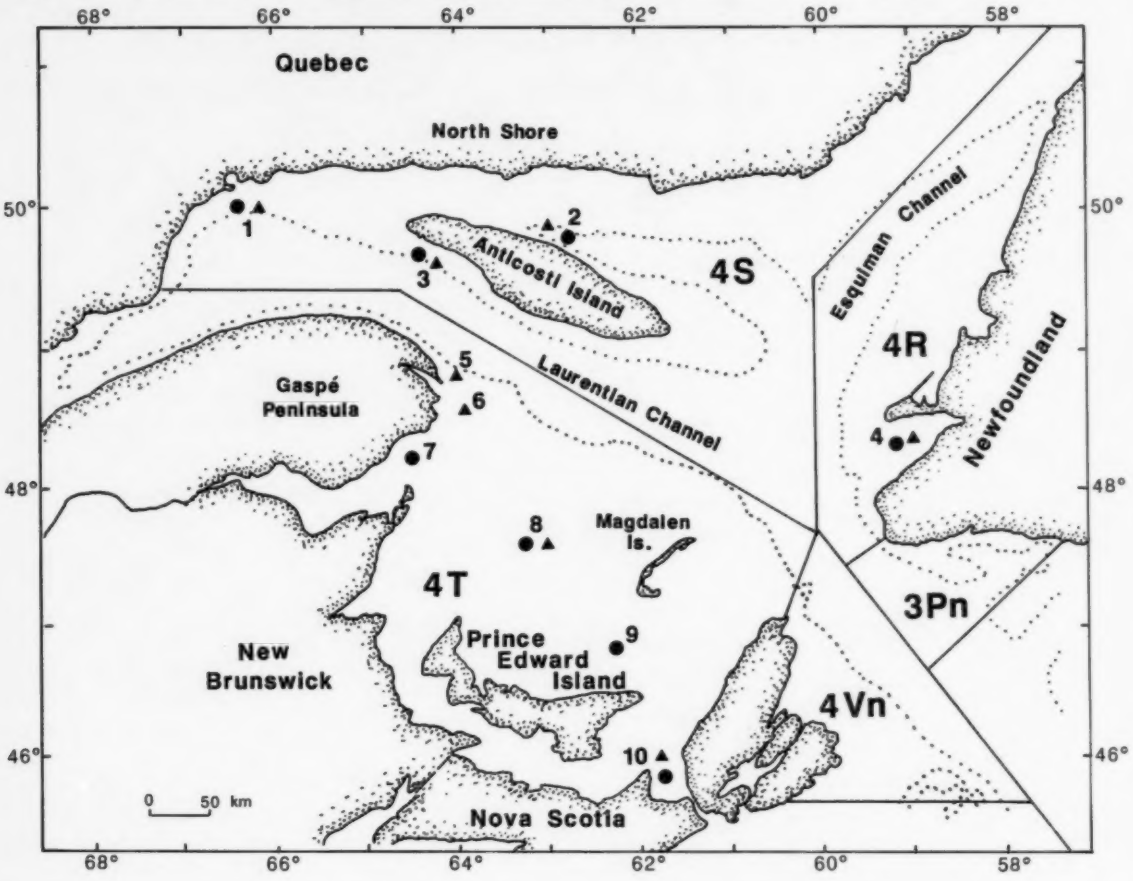
Fig. 1. Location of samples of Atlantic cod (*Gadus morhua*) (●) and American plaice (*Hippoglossoides platessoides*) (▲) in the Gulf of St. Lawrence. 1, North Shore; 2, Northern Anticosti; 3, Western Anticosti; 4, Southwest Newfoundland; 5, Northern Gaspé (A); 6, Northern Gaspé (B); 7, Southern Gaspé; 8, Bradelle Bank; 9, Prince Edward Island; 10, St. Georges Bay.



and flatfish in eastern Canada in light of rapidly increasing grey seal (*Halichoerus grypus*) populations. Grey seals appear to be the most important definitive hosts of sealworm (Mansfield and Beck 1977; McClelland 1980; McClelland et al. 1983*a*; Brattey and Stenson 1993). With the exception of plaice surveys (McClelland et al. 1987) restricted to fish 31–40 cm in length, infections in fish from both the northern and southern Gulf of St. Lawrence (NAFO divisions 4RST) have not been investigated simultaneously for three decades.

This study analyses geographical variations in abundances of *P. decipiens*, *A. simplex*, and contracaecines in Atlantic cod and American plaice from the Gulf of St. Lawrence and compares recent infection levels with those recorded in earlier surveys.

Materials and methods

Fish were collected from various sites in the Gulf of St. Lawrence in 1990 and 1992 during Department of Fisheries and Oceans scientific missions and from commercial catches (Fig. 1). Although the precise location of the commercial sets was unknown, the corresponding NAFO subdivisions were provided by fishermen. Fish were measured and selected according to an orthogonal sampling design. Cod were stratified into twelve, 5-cm length classes (≤20, 21–25, 26–30, 31–35, 36–40, 41–45, 46–50, 51–55, 56–60, 61–65, 66–70, ≥71) containing equal number of fish, whenever possible. Plaice were stratified into eight 5-cm length groups (≤20, 21–25, ..., ≥51). In many cases, it was not possible to conform to the sampling design and fill the smaller and larger length categories because of the type of gear used and differential length-class strength. Fish from commercial samples were of random lengths, and small fish were often lacking.

Fish samples collected from research vessels were frozen at sea. Those collected from commercial vessels were stored on ice for transport to the fish plant, where they were measured and frozen. All fish were stored at −20°C until examination. After thawing, each fish was measured to the nearest millimetre, weighed to the nearest gram, gutted, sexed, and filleted. The flesh was inspected by systematic destruction

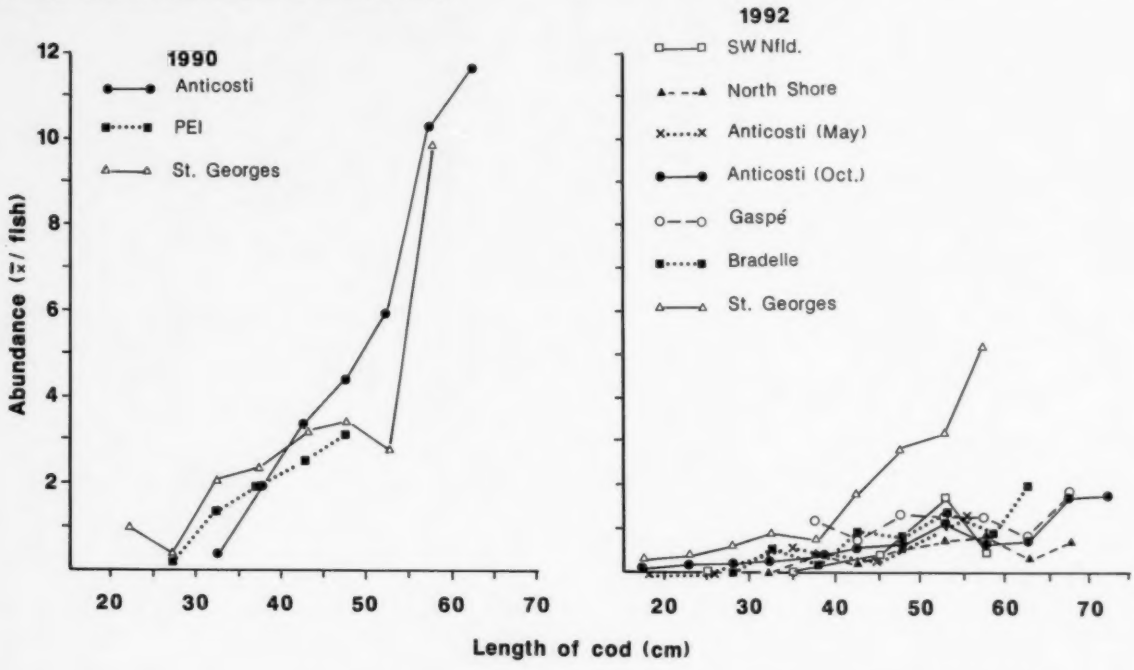
Table 1. Geographical variations in prevalence (*P*), abundance (*A* ± SE), and maximum intensity (MI) of larval anisakines in Atlantic cod (*Gadus morhua*) from the Gulf of St. Lawrence.

Sample areas (month/year)	NAFO subdivisions	*N*	Mean length (cm)	*P. decipiens*					*A. simplex*					Contracaecinea				
				P (%)	*A*	(±SE)	MI	Group*	*P* (%)	*A*	(±SE)	MI	Group*	*P* (%)	*A*	(±SE)	MI	Group*
Northern Anticosti (9/90)	4Sx	211	35.1	44.5	2.20	(0.26)	22	*b,c*	38.4	3.57	(1.25)	256	*b,c*	36.0	5.97	(0.98)	108	*b,†c*
Western Anticosti (5/92)	4Ss	83	31.3	16.9	0.30	(0.08)	3	*e*	42.2	1.86	(0.35)	16	*b,c*	34.9	2.99	(0.92)	61	*c,d*
Western Anticosti (10/92)	4Ss	255	44.1	33.3	0.62	(0.07)	6	*e*	62.4	6.70	(0.76)	118	*a*	57.6	12.68	(1.46)	123	*a*
North Shore (9/92)	4Sz	131	50.7	33.6	0.56	(0.08)	6	*e*	88.5	4.53	(0.46)	41	*a*	74.0	5.24	(0.91)	75	*a,b*
Southwest Newfoundland (5/92)	4Rd	75	45.9	20.0	0.45	(0.12)	6	*e*	68.0	6.16	(1.53)	70	*a*	54.7	6.97	(1.46)	65	*a,b*
Southern Gaspé (6/92)	4Tn	110	50.2	58.2	1.19	(0.12)	4	*c,d*	59.1	1.60	(0.19)	9	*b,c*	20.9	1.14	(0.32)	22	*d,e,f*
Bradelle Bank (9/92)	4Tk	118	45.0	41.5	0.81	(0.11)	4	*d,e*	67.8	2.12	(0.27)	17	*b*	30.5	2.31	(0.74)	63	*d,e*
Prince Edward Island (9/90)	4Tg	90	38.8	78.9	2.04	(0.19)	10	*a,b*	44.4	0.89	(0.17)	9	*c,d*	7.8	0.22	(0.15)	13	*f*
St. Georges Bay (9/90)	4Tg	115	39.6	81.7	2.94	(0.33)	28	*a*	18.3	0.50	(0.18)	18	*d*	13.0	0.26	(0.08)	6	*e,f*
St. Georges Bay (9/92)	4Tg	137	37.6	56.9	1.57	(0.19)	15	*b,c*	29.9	0.74	(0.14)	12	*d*	9.5	0.52	(0.26)	31	*f*

*Tukey's studentized range test on nematode abundances computed from $\log_{10}(x + 1)$ transformed parasite counts. Abundances with the same letters are not significantly different at $\alpha = 0.01$. This test controls the type I experimentwise error rate. Abundances with more than one letter result from the inability of the test to determine to which group the sample belongs (Zar 1984).

†The Northern Anticosti sample was significantly different from North Shore but not from Southwest Newfoundland.

Fig. 2. Abundance of sealworm (*Pseudoterranova decipiens*) in relation to length class of Atlantic cod (*Gadus morhua*) from various sites in the Gulf of St. Lawrence (4RST).



(Power 1961). The viscera were inspected with a magnifying lens. Nematodes found in the musculature, on the viscera, and in the mesenteries were identified and counted. *Contracaecum osculatum* (Rudolphi, 1802) (probably *Contracaecum osculatum* B according to Brattey and Stenson 1993) and *Phocascaris* sp. were grouped under the tribe Contracaecinea because it was not possible to separate these two species according to anatomical characteristics (Berland 1961; Fagerholm 1988; Likely and Burt 1992).

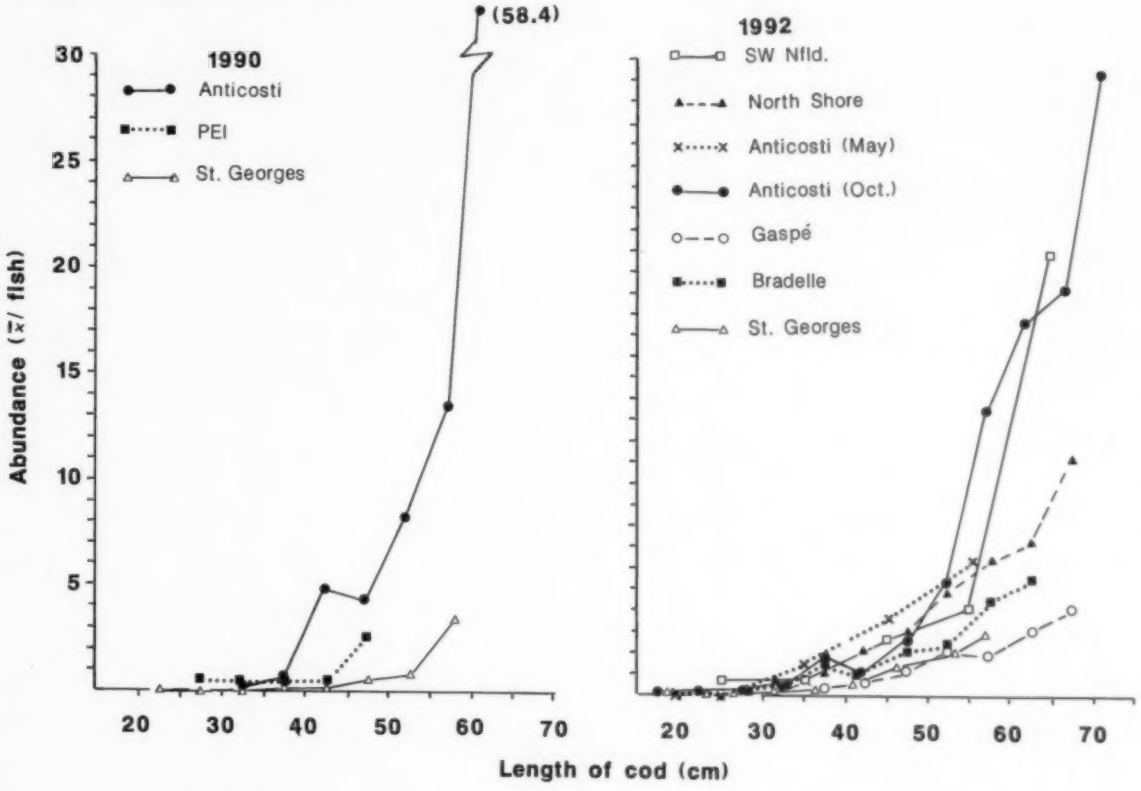
Prevalence is the percentage of fish infected with a given parasite species in a sample, abundance is the mean number of parasites of a particular species per fish examined, and maximum intensity is the highest number of parasites of a given species recorded in an infected host (Margolis et al. 1982). Counts of larval anisakines in each fish were transformed using $\log_{10}(x + 1)$, bringing positively skewed frequency distributions of anisakines closer to normality (Platt 1975; McClelland et al. 1983*a*, 1983*b*, 1985, 1987). To reduce disparities in cell frequencies and the numbers of blank cells, cod were partitioned into ≤35, 36–45, 46–55, and ≥56 cm length classes, and plaice into classes of ≤20, 21–30, 31–40, and ≥41 cm. Because blank cells could not be eliminated, variations in abundances of larval anisakines with fish length and sampling location (sample) were analyzed using a Type IV two-way ANOVA (GLM procedure, SAS Institute Inc. 1988), for each parasite and fish species, where applicable. Host sex was dropped from the ANOVAs because preliminary analyses indicated nonsignificant effects on parasite abundances. When the overall *F*-test revealed significant geographical variation in abundance of a given anisakine species, Tukey's studentized range test was used to determine which sample means differed significantly at $\alpha = 0.01$. Temporal variations in anisakine abundances were investigated statistically in cod using samples collected in 1990 and 1992 from St. Georges Bay, Nova Scotia. Other temporal trends in abundance of anisakine nematodes in cod and plaice were assessed by comparing recent infection levels with those reported in earlier surveys (McClelland et al. 1983a, 1985, 1987) using fish of similar sizes sampled at corresponding sites but were not tested statistically.

Results

Anisakines in Atlantic cod

Pseudoterranova decipiens was present in all samples and was the most abundant anisakine nematode in cod from the southern Gulf of St. Lawrence (4T) (Table 1). Abundance of *P. decipiens* varied significantly between samples ($F = 68.08$, df = 9, $p \leq 0.0001$) (Table 1). Infection levels were highest in southern Gulf (4T) cod but generally lower to the north and in offshore areas of this zone (Table 1; Fig. 2). Cod collected north of the Laurentian Channel (4RS), with the exception of those sampled north of Anticosti Island in 1990, had lower sealworm levels than 4T cod (Table 1; Fig. 2). Abundance of *P. decipiens* generally increased with host size (Fig. 2). Variations in abundance of *P. decipiens* with host size were highly significant ($F = 77.71$, df = 3, $p \leq 0.0001$), but differed among samples as indicated by the significant host length–sample interaction ($F = 9.22$, df = 26, $p \leq 0.0001$) (Fig. 2). In St. Georges Bay, abundance of *P. decipiens* was significantly

Fig. 3. Abundance of whaleworm (*Anisakis simplex*) in relation to length class of Atlantic cod (*Gadus morhua*) from various sites in the Gulf of St. Lawrence (4RST).



greater in cod sampled in 1990 than in those taken in 1992 (Table 1).

Unlike sealworm, *Anisakis simplex* was most abundant in the northern Gulf (4RS) (Table 1). Geographical differences in abundances of *A. simplex* were significant (F = 31.60, df = 9, $p \leq 0.0001$) (Table 1). Infection levels were lower in 4T cod, and inshore cod tended to have lighter infections than offshore cod (Table 1; Fig. 3). Abundance of *A. simplex* varied significantly with host size (F = 202.32, df = 3, $p \leq 0.0001$), large cod being generally more infected than smaller ones (Fig. 3). The relationship between abundance of *A. simplex* and length of cod varied significantly among samples, as shown by the host length–sample interaction (F = 10.76, df = 26, $p \leq 0.0001$) (Fig. 3). Infection levels in cod from St. Georges Bay did not change significantly between 1990 and 1992 (Table 1).

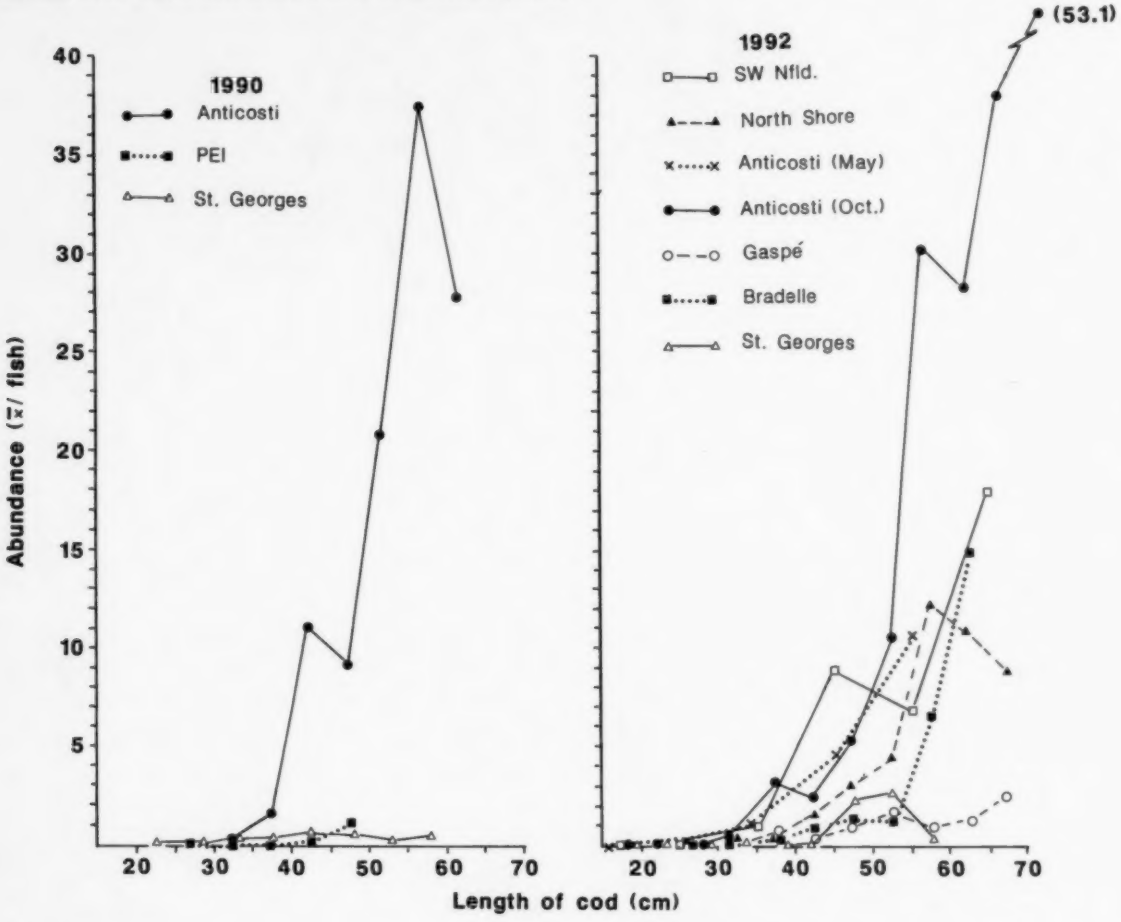
Geographical differences in abundances of contracaecines in Gulf (4RST) cod generally resembled those of *A. simplex* and were highly significant (F = 57.06, df = 9, $p \leq 0.0001$) (Table 1). Infection levels were highest in northern Gulf (4RS) cod and lowest in the southernmost cod samples (Table 1; Fig. 4). Abundances of contracaecines increased with host length (Fig. 4). The trend was highly significant (F = 111.83, df = 3, $p \leq 0.0001$) but varied significantly among samples (F = 14.06, df = 26, $p \leq 0.0001$) (Fig. 4). Cod from St. Georges Bay were more heavily infected in 1992 than in 1990 but differences in abundance were not significant (Table 1).

Anisakines in American plaice

Infection levels of anisakines in plaice were much lower than those in cod. Larval sealworm was the most abundant anisakine in plaice. Abundance of *P. decipiens* varied significantly (F = 15.56, df = 8, $p \leq 0.0001$) with sampling location (Table 2; Fig. 5) and was highest in plaice from the southern Gulf (4T) (Table 2). However, significant local variations were observed in plaice from Northern Gaspé (4Tn) (Table 2). Abundance of *P. decipiens* varied significantly with host length (F = 7.75, df = 3, $p \leq 0.0001$) and in regard to host length–sample interaction (F = 3.14, df = 21, $p \leq 0.0001$) (Fig. 5).

Abundances of *A. simplex* and contracaecines were low in plaice (Table 2) but were generally higher in plaice from the northern Gulf (4RS) than in those from the southern Gulf (4T) (Table 2). Analysis revealed significant variations in abundance of *A. simplex* with host length (F = 8.60, df = 3, $p \leq 0.0001$). Differences in abundance of *A. simplex* with geographical origin of sample (F = 1.88, df = 8, p = 0.0603) (Table 2), and with the sample-host length interaction (F = 0.97, df = 21, p = 0.5033), were not significant. Abundances of contracaecines were not analysed because infection levels were too low.

Fig. 4. Abundance of Contracaecinea (*Contracaecum*/*Phocascaris* spp.) in relation to length class of Atlantic cod (*Gadus morhua*) from various sites in the Gulf of St. Lawrence (4RST).



Discussion

Distribution of *Pseudoterranova decipiens* in cod and plaice from the Gulf of St. Lawrence

Paggi et al. (1991) describe three sibling species of *P. decipiens* in the North Atlantic based on biochemical evidence, and Bristow and Berland (1992) caution against acceptance of previous surveys such as McClelland et al. (1983*a*, 1983*b*) where structurally similar but reproductively isolated sibling species have not been separated. However, only *P. decipiens* B was found in fish and seals from the Gulf of St. Lawrence (Paggi et al. 1991; Brattey and Stenson 1993). Sibling species A did not occur in North American waters, and type C infects only bearded seals, *Erignathus barbatus*, which are seldom found in the Gulf of St. Lawrence. Thus, sealworm detected in the Gulf and in waters off Nova Scotia are probably type B (McClelland and Marcogliese 1994).

Our study showed important geographical variations in the abundance of sealworm among fish samples from the Gulf of St. Lawrence (4RST). Samples from the southern Gulf (4T) were generally more heavily infected than those north of the Laurentian Channel (4RS). Similar broad geographical variations in the infection levels of sealworm in cod and plaice were documented by Templeman et al. (1957), Scott and Martin (1957), and McClelland et al. (1985, 1987), although McClelland et al. (1985, 1987) reported increasing infection levels in cod and flatfish from both the southern (4T) and northern (4RS) parts of the Gulf. In 1992, abundances of *P. decipiens* did not vary significantly in cod and plaice samples from the northern Gulf (4RS), but some variations occurred in samples from the southern Gulf (4T), with infections being higher in inshore than in offshore fish. Infection patterns of *P. decipiens* in cod likely correspond to Gulf cod stocks. The cod stocks (4RS-3Pn and 4T-Vn) are separated by the Laurentian Channel (Clay 1991; Chouinard and Fréchet 1993), but many studies suggest that cod from the eastern and western portions of the southern Gulf do not form a unit stock (McKenzie and Smith 1955; Martin and Jean 1964; Lambert 1993; McClelland and Marcogliese 1994; Campana et al. 1995). This could explain, in part, variations of sealworm

infections in cod between the northern and southern Gulf, and within the southeastern and southwestern Gulf. In addition, higher abundances of sealworm in inshore cod may be due to the proximity of seal colonies (Young 1972; McClelland et al. 1987; Jensen and Idås 1992; Marcogliese and McClelland 1992).

Comparisons of our results with earlier records suggest temporal changes in abundance of sealworm. Sealworm abundance observed in cod from St. Georges Bay (4T) in 1990 appeared higher than that observed by McClelland et al. (1983*a*) and was significantly greater than that found in the 1992 sample. The apparent increase in abundance of sealworm between 1983 and 1990 was probably a result of growth of the grey seal herd in the southeastern Gulf (Zwanenburg and Bowen 1990), but the apparent decline in sealworm abundance between 1990 and 1992 is puzzling. A similar decline in sealworm abundance between 1990 and 1992 was observed in longhorn sculpin (*Myoxocephalus octodecemspinosus*) and white hake (*Urophycis tenuis*) from St. Georges Bay (Marcogliese 1995).

In the northern Gulf, infection levels of sealworm in cod are slightly higher than those of McClelland et al. (1985) in 1984, but comparisons are difficult because of differences in the breakdown of size classes. The 1990 cod sample from northern Anticosti was exceptional in that sealworm abundance was comparable to abundances observed in 1990 from the southern Gulf (4T). Sealworm levels were also comparatively high in shorthorn sculpin (*Myoxocephalus scorpius*) and Greenland halibut (*Reinhardtius hippoglossoides*) from northern Anticosti in 1990 (Marcogliese 1995). However, different fish populations may have been sampled within the northern Gulf in 1990 and 1992. It is unlikely that sealworm abundance declined to such a great extent in two years, as sealworm is reputed to be long lived in fish hosts.

Abundance of sealworm in plaice varied throughout the Gulf of St. Lawrence. Plaice from the northern Gulf had somewhat lighter infections than those from the southern Gulf. However, significant local variation was detected. Infection levels in plaice may be indicative of the abundances of sealworm in discrete geographical areas because plaice are more sedentary than cod (McClelland et al. 1985).

No consistent temporal trends in sealworm abundance in plaice were observed in either the southern or northern Gulf since 1980 (McClelland et al. 1983a, 1985, 1987; this study), although McClelland et al. (1987) reported significant increases in sealworm abundances at four of seven sites in the Gulf. Inconsistent variations observed since 1980 may have been local spatial variations. Causes of the local differences in sealworm abundance among plaice are presently unknown. Regardless, it appears that local variations of sealworm in sedentary fish such as American plaice can be quite high. Comprehension of this phenomenon requires repeated sampling at a much finer scale than employed in this or other studies.

Water temperature may also influence the geographical distribution of sealworm in fish (Templeman et al. 1957) and can affect the hatching and development of sealworm in poikilothermic hosts (McClelland 1982; Brattey 1990; Burt et al. 1990). Mean bottom (about 30 m) temperatures

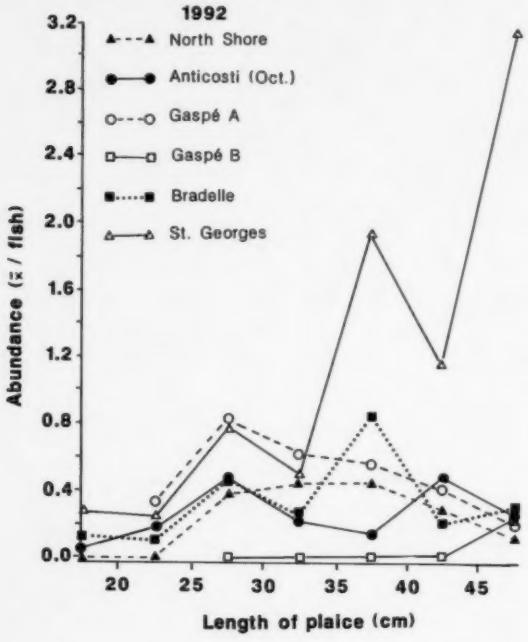
Table 2. Geographical variations in prevalence (*P*), abundance (*A* ± SE), and maximum intensity (MI) of larval anisakines in American plaice (*Hippoglossoides platessoides*) from the Gulf of St. Lawrence.

Sample areas (month/year)	NAFO subdivisions	*N*	Mean length (cm)	*P. decipiens*					*A. simplex*[†]				Contracaecinea			
				P (%)	*A*	(±SE)	MI	Group*	*P* (%)	*A*	(±SE)	MI	*P* (%)	*A*	(±SE)	MI
Northern Anticosti (9/90)	4Sx	121	24.3	9.9	0.20	(0.06)	4	*c,d*	4.1	0.07	(0.04)	5	3.3	0.10	(0.06)	6
Western Anticosti (5/92)	4Ss	100	27.5	0.0	0.00	(0.00)	0	*d*	7.0	0.12	(0.05)	4	1.0	0.02	(0.02)	2
Western Anticosti (10/92)	4Ss	129	31.1	14.7	0.26	(0.07)	4	*c,d*	4.7	0.09	(0.04)	4	0.8	0.03	(0.03)	4
North Shore (9/92)	4Sz	127	35.9	18.9	0.25	(0.05)	3	*c,d*	7.9	0.10	(0.03)	2	0.0	0.00	(0.00)	0
Southwest Newfoundland (5/92)	4Rd	100	28.4	0.0	0.00	(0.00)	0	*d*	2.0	0.03	(0.02)	2	2.0	0.02	(0.01)	1
Northern Gaspé (sample A) (7/92)	4Tn	105	37.1	38.1	0.52	(0.07)	2	*a,b*	10.5	0.12	(0.04)	2	0.0	0.00	(0.00)	0
Northern Gaspé (sample B) (6/92)	4Tn	105	41.9	5.7	0.09	(0.04)	2	*c,d*	3.8	0.06	(0.03)	3	0.0	0.00	(0.00)	0
Bradelle Bank (9/92)	4Tk	160	34.5	20.0	0.35	(0.07)	5	*b,c*	1.9	0.03	(0.02)	2	0.0	0.00	(0.00)	0
St. Georges Bay (9/92)	4Tg	124	30.4	40.3	0.89	(0.16)	15	*a*	2.4	0.02	(0.01)	1	0.8	0.01	(0.01)	1

*Tukey's studentized range test on sealworm (*Pseudoterranova decipiens*) abundances computed from $\log_{10}(x + 1)$ transformed parasite counts. Abundances with the same letters are not significantly different at $\alpha = 0.01$. This test controls the type I experimentwise error rate. Abundances with more than one letter result from the inability of the test to determine to which group the sample belongs (Zar 1984).

†The effect of geographical location was not significant ($p = 0.0603$) in a preliminary ANOVA.

Fig. 5. Abundance of sealworm (*Pseudoterranova decipiens*) in relation to length class of American plaice (*Hippoglossoides platessoides*) from various sites in the Gulf of St. Lawrence (4RST).



in St. Georges Bay are much higher than the rest of the Gulf in summer and fall (Petrie 1990), and this could lead to rapid hatching and development of sealworm larvae in that area (McClelland 1982; Brattey 1990).

Distribution of *Anisakis simplex* in cod and plaice from the Gulf of St. Lawrence

Anisakis simplex is the most cosmopolitan member of the genus and is frequently found in fish from cold temperate and polar waters (Young 1972; Platt 1975; Smith and Wootten 1978; McClelland et al. 1983*a*; McGladdery 1986; Brattey and Bishop 1992). Genetic evidence suggests the existence of two sibling species (Orecchia et al. 1986), of which type B appears to be most common off North America (Brattey and Clark 1992).

The geographical distribution of *A. simplex* in cod from the Gulf is markedly different from that of sealworm. The highest and lowest infection levels of *A. simplex* occurred in samples collected north of the Laurentian Channel, and in the southern Gulf (4T), respectively. Marcogliese (1995) observed similar patterns for longhorn and shorthorn sculpin, witch flounder (*Glyptocephalus cynoglossus*), and Greenland halibut. In general, infection levels of *A. simplex* were similar among cod samples within the northern Gulf (4RS) and among those within the southern Gulf (4T). In the southern Gulf (4T), offshore samples of cod tended to be more heavily infected than inshore ones. Infection levels of *A. simplex* have remained relatively constant in the Gulf since 1981, with infections being lowest in the southern Gulf inshore, higher in the southern Gulf offshore, and extremely high in the northern Gulf (McClelland et al. 1983*a*, 1985; this study).

Plaice were lightly infected compared to cod but, like cod, they were more heavily infected in the northern Gulf than in the southern Gulf. However, the geographical variations in abundance of *A. simplex* in plaice were not significant because of relatively low sample sizes and infection levels. Low infection levels in plaice (see also Templeman et al. 1957; McClelland et al. 1983*a*, 1983*b*, 1985) suggest that this benthic fish is not an important host of whaleworm. As with sealworm, plaice caught off northern Gaspé in July 1992 were more heavily infected with whaleworm than those caught nearby. This further demonstrates the variability of infection levels in neighbouring plaice populations.

Temporal trends in infection levels of *A. simplex* in plaice differ between regions of the Gulf. In the southeastern Gulf, infection levels of *A. simplex* in plaice have remained extremely low since 1980 (McClelland et al. 1983a; this study). The highest prevalence and abundance of whaleworm in the northern Gulf was observed in Esquiman Channel in 1984 (McClelland et al. 1985).

The geographical and temporal distribution of *A. simplex* in fish can only be interpreted in general terms, as we know little about the distribution, abundance, and infection levels of the main definitive hosts, the cetaceans (Smith 1983; Smith and Wootten 1978; McClelland et al. 1985; Brattey and Bishop 1992). The parasite seldom matures in seals off Atlantic Canada (Brattey and Stenson 1993). High levels of *A. simplex* in cod from the northern Gulf can be partly attributed to the presence of numerous whales in this region during the ice-free period.

Distribution of Contracaecinea in cod and plaice from the Gulf of St. Lawrence

Abundances of contracaecines varied geographically and with host size. Contracaecines were the most abundant anisakines in cod from the northern Gulf. They were much less common in the southern Gulf, despite the winter presence of a large herd of harp seals (*Phoca groenlandica*) off the Magdalen Islands. The harp seal is believe to be the most important final host of C. *osculatum* (McClelland et al. 1985; Brattey and Ni 1992) and the parasite is abundant in ice-breeding harp seals from the southern Gulf (Myers 1957; Scott and Fisher 1958). Unlike in cod, these nematodes were rare in corresponding plaice samples. Cod acquire heavy anisakine infections when they become piscivorous (McClelland et al. 1983*a*). As the geographical distribution of contracaecines in cod and plaice is generally consistent with that of *A. simplex*, but not with that of sealworm, it is possible that *Contracaecum* is transmitted to fish by pelagic prey, as is *Anisakis* (McClelland et al. 1990). In the northern Gulf, capelin (*Mallotus villosus*) are an important food resource for cod (Rose and Leggett 1989) and can be an important intermediate or paratenic host for both *Contracaecum* sp. and *A. simplex* (Pálsson and Beverley-Burton 1984; McClelland et al. 1985; Marcogliese 1995); however, they are not infected with sealworm (Margolis and Arthur 1979; Pálsson and Beverley-Burton 1984; Marcogliese and McClelland 1992; Marcogliese 1995). Moreover, capelin are seldom found

in the southern Gulf (Parent and Brunel 1976). Alternatively, as species of *Contracaecum* and *Phocascaris* occur primarily in arctic or subarctic seals (Brattey 1990), their distributions in the Gulf of St. Lawrence may reflect adaptations to cold temperatures.

In this survey, abundances of contracaecines in cod were much higher in the southern Gulf, and somewhat higher in the northern Gulf, than previously reported (McClelland et al. 1983*a*, 1985). Apparent increases in levels of contracaecines in cod between 1981 and 1992 are probably due to population growth of grey (Zwanenburg and Bowen 1990) and harp seals (Stenson et al. 1993) in the Gulf of St. Lawrence.

Acknowledgments

We thank Jean-Denis Lambert for coordinating the in-plant sampling program, and Monique Tapp for examining the fish. We also gratefully acknowledge Élaine Albert, Bernard Chenard, Johanne Guérin, Chris Keeling, Lena Measures, François Roy, Dick Russell, and the officers and crews of the research vessels *E.E. Prince* and *Alfred Needler* for their assistance in the sampling program, and Pierre Gagnon for statistical advice. Comments provided by Gary McClelland greatly improved the manuscript and are gratefully acknowledged. Funding was provided by the Programme Fédéral de Développement des Pêches du Québec.

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Identification of larval *Contracaecum osculatum* s.l. and *Phocascaris* sp. (Nematoda: Ascaridoidea) from marine fishes by allozyme electrophoresis and discriminant function analysis of morphometric data

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Abstract: Biochemical markers (allozymes) were used to identify third-stage larvae of nematodes from the *Contracaecum osculatum* s.l. – *Phocascaris* sp. complex found in five marine fish species (*Hippoglossoides platessoides*, *Gadus morhua*, *Mallotus villosus*, *Myoxocephalus scorpius*, *Pleuronectes americanus*) collected off Newfoundland and Labrador. Three taxonomic groups, genetically identical to adult *C. osculatum* A, *C. osculatum* B, and *Phocascaris* sp. previously reported from North Atlantic seals, were found. Among the 859 larvae examined, *C. osculatum* B was most common (74.2%), particularly in *H. platessoides* (79.4% of 501 larvae), *G. morhua* (88.8% of 160), *M. scorpius* (87.5% of 32), and *P. americanus* (100.0% of 20), but *Phocascaris* sp. was more numerous in *M. villosus* (66.4% of 146). *Contracaecum osculatum* A was found only in *H. platessoides* (15% of 501). Discriminant function analysis revealed differences in shape among the nematode species. Larvae of *Phocascaris* sp. and *C. osculatum* B can generally be distinguished using three measurements and classified according to coefficients for quadratic discriminant functions, but larvae of *C. osculatum* A could not be reliably distinguished from the other two species. A simpler, more rapid method of screening biochemical markers, employing a cellulose acetate support medium, is also described.

Résumé : Des marqueurs biochimiques (allozymes) ont servi à identifier des larves de troisième stade de nématodes du complexe *Contracaecum osculatum* s.l. – *Phocascaris* sp. retrouvées chez cinq espèces de poissons marins (*Hippoglossoides platessoides*, *Gadus morhua*, *Mallotus villosus*, *Myoxocephalus scorpius*, *Pleuronectes americanus*) capturés dans les eaux de Terre-Neuve et du Labrador. Nous avons repéré trois groupes taxinomiques, génétiquement identiques aux spécimens adultes de *C. osculatum* A, *C. osculatum* B et *Phocascaris* sp. déja signalés chez les phoques de l'Atlantique Nord. Parmi les 859 larves examinées, le *C. osculatum* B était le nématode le plus commun (74,2%), particulièrement chez *H. platessoides* (79,4% des 501 larves), *G. morhua* (88,8% des 160 larves), *M. scorpius* (87,5% des 32 larves) et *P. americanus* (100,0% des 20 larves), mais les *Phocascaris* sp. étaient plus nombreux chez *M. villosus* (66,4% des 146 larves). Le *Contracaecum osculatum* A était présent seulement chez *H. platessoides* (15% des 501 larves). L'analyse discriminante a révélé des différences de taille entre les espèces de nématodes. Les larves de *Phocascaris* sp. et de *C. osculatum* B peuvent en général se différencier par trois mesures et se classer selon les coefficients donnés aux fonctions quadratiques discriminantes, mais les larves de *C. osculatum* A n'ont pas pu être de façon fiable distinguées de celles des deux autres espèces. Nous décrivons aussi une méthode plus simple et plus rapide de différenciation des marqueurs biochimiques à l'aide d'un milieu à base d'acétate de cellulose.

[Traduit par la Rédaction]

Received November 29, 1993. Accepted December 15, 1994.
J12666

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Can J. Fish. Aquat. Sci. **52**(Suppl. 1): 116–128 (1995). Printed in Canada / Imprimé au Canada

Introduction

Parasitic nematodes of the genera *Contracaecum* Raillet and Henry, 1912 and *Phocascaris* Host, 1932 are common in the gastrointestinal tract of seals in the North Atlantic (Lyster 1940; Myers 1957*a*, 1957*b*; Scott and Fisher 1958; Pálsson 1977; Fagerholm 1989; Brattey and Ni 1992; Brattey and Stenson 1993). Their life cycles are poorly known, but adults inhabit the stomach or anterior intestine of seals and female nematodes shed eggs that are released with the host's faeces. The eggs develop and hatch, releasing ensheathed larvae that are ingested by and infect crustaceans (Davey 1969; Brattey 1990). Larvae are transferred to fish and subsequently to seals during predator–prey interactions and in seals they develop to maturity.

There are numerous records of *Contracaecum*-type larvae in marine fishes from the North Atlantic (Berland 1961; Ronald 1963; Wootten 1978; McClelland et al. 1983, 1985; Pálsson and Beverley-Burton 1984). However, the genus *Contracaecum* includes several species with different life cycles and includes types that mature in the digestive tract of birds (Huizinga 1966), marine mammals (Berland 1963), or fishes (Deardorff and Overstreet 1981). Their larvae are morphologically similar and the taxonomy of the larval forms is confused (see Berland 1961; Deardorff and Overstreet 1981; Soleim 1984). Many of the early records of larval *Contracaecum* spp. from Canadian marine fishes probably involve larval *Hysterothylacium* spp. (formerly *Thynnascaris* spp., *C. clavatum* (Rudolphi, 1809), or *C. aduncum* (Rudolphi, 1802)) (see Margolis and Arthur 1979), which matures in the gastrointestinal tract of piscivorous fishes (Deardorff and Overstreet 1981). Others are possibly larvae of *C. osculatum* (Rudolphi, 1802) from seals, but according to Berland (1963) the third-stage larvae of *C. osculatum* and *Phocascaris* sp. are almost identical and reports of larval *C. osculatum* from Canadian marine fishes possibly include both species. Consequently, the literature on parasites of marine fishes contains many records of larval *Contracaecum* spp. with unknown life cycles.

Before the mid-1980s conclusive identification of the third-stage larvae of *C. osculatum* from marine fishes had proven difficult. *Contracaecum*-type larvae from Atlantic cod (*Gadus morhua*) of Baltic origin were identified as *C. osculatum* following surgical implantation and maturation in the body cavity of rats (Fagerholm 1988). McClelland and Ronald (1974) also reared *C. osculatum* from egg to "preadults" in vitro and later (McClelland et al. 1985) found that the cultured larvae closely resembled *Contracaecum*-type larvae found in Canadian Atlantic cod and American plaice, *Hippoglossoides platessoides*. Likely and Burt (1992) confirmed McClelland et al.'s (1985) suggestion by rearing larvae of *Contracaecum* sp. from Canadian Atlantic cod to sexually mature adults in vitro and concluded that they were *C. osculatum*. As adults, *C. osculatum* and *Phocascaris* sp. can be distinguished easily by examining their cephalic structures (Host 1932; Lyster 1940; Berland 1963; Fagerholm 1989). However, the third-stage larvae of *C. osculatum* and *Phocascaris* sp. are much more difficult to distinguish (Berland 1963; Smith and Wootten 1984).

More recently, genetic studies based on allozymes have shown that *Contracaecum osculatum* infecting phocids in the North Atlantic and adjacent areas consists of at least three genetically distinct taxa, with no evidence of hybridization between them (Nascetti et al. 1986*a*, 1993; Orecchia et al. 1986*a*). In Canadian Atlantic phocids, two species within *C. osculatum* have been found, presently designated A and B. Adults of *C. osculatum* A occur almost solely in the bearded seal, *Erignathus barbatus*, whereas *C. osculatum* B is found predominantly in the harp seal, *Phoca groenlandica*, but also in five other seal species (Brattey and Ni 1992; Brattey and Stenson 1993). The genetic studies have also provided reliable biochemical markers that allow *C. osculatum* A, *C. osculatum* B, and *Phocascaris* sp. to be distinguished from one another.

Studies on the biology of third-stage larvae of *Contracaecum osculatum* and *Phocascaris* sp. from North Atlantic marine fishes have been hindered by the lack of a reliable and simple means of distinguishing them. The objectives of the present study were therefore (*i*) to determine how many species of larvae within the *C. osculatum* – *Phocascaris* sp. complex occur in marine fishes off Newfoundland and Labrador, (*ii*) to provide preliminary information on their geographic distribution and host preferences, and (*iii*) to find simpler methods for distinguishing them. Morphometric measurements of larvae from various marine fish species were obtained and specimens subsequently identified using biochemical markers. These data were used in an attempt to develop discriminant functions (DFs) that could be used to classify the larvae. A simpler more rapid method of screening diagnostic biochemical markers (allozymes) is also described.

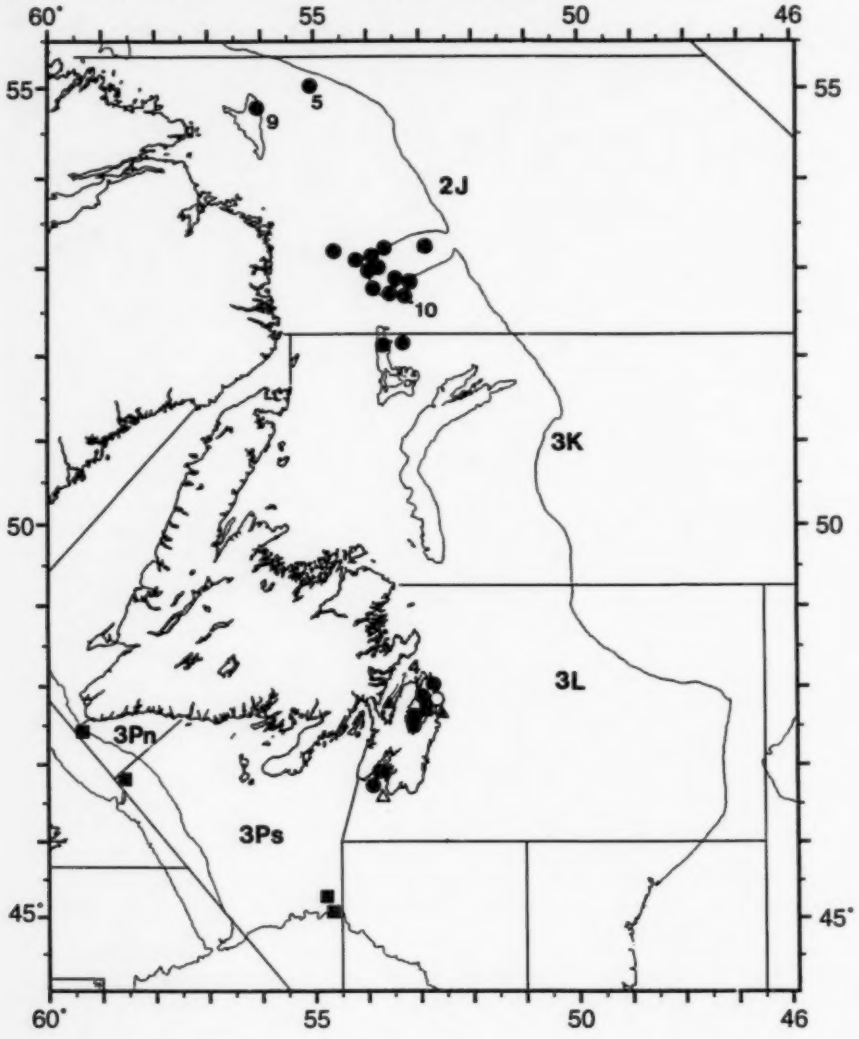
Materials and methods

Collection of samples

Larval nematodes were obtained from five marine fish species collected at various locations around Newfoundland and Labrador during 1991–1992 (Fig. 1, Table 1). Fish were collected with an Engels 145 bottom otter trawl, fitted with a 29-mm mesh liner in the codend, on Department of Fisheries and Oceans (DFO) research cruises. One subsample, comprised of 5–15 fish of one species, was retained for examination from individual sets. Additional samples of Atlantic cod and capelin (*Mallotus villosus*), each comprised of 50–100 fish, were obtained from cod-traps and spawning beaches, respectively, and examined for nematodes.

Most samples were examined on board the vessel on the day of capture but some samples, including those from cod traps and spawning beaches, were initially stored on ice for 1–3 d before examination. Live nematodes were recovered from the viscera and body cavity of fish by visual inspection, pepsin – hydrochloric acid (HCl) digestion (Stern et al. 1958), or a combination of both methods. Flesh was not examined. Nematodes were washed in distilled water and stored live in chilled (2°C) saline for up to 3 weeks. Specimens of *Contracaecum osculatum* – *Phocascaris* sp. were distinguished from other marine ascaridoids (i.e., *Anisakis*, *Pseudoterranova*, *Hysterothylacium*) using anatomical features (see Berland 1961, 1963; Deardorff and Overstreet 1981). Many (58.3%) of the specimens were collected from American plaice because preliminary

Fig. 1. Newfoundland and southern Labrador showing Northwest Atlantic Fisheries Organization (NAFO) boundaries (solid lines) and set locations: solid circles, *Hippoglossoides platessoides*; solid squares, *Gadus morhua*; solid triangles, *Mallotus villosus*; open circle, *Myoxocephalus scorpius*; open triangle, *Pleuronectes americanus*. Numbers adjacent to symbols are coincident sets. Depth contour is 400 m.



sampling revealed that plaice were infected with all the nematode species under investigation (see Results).

Adult nematodes were obtained from the stomach or anterior small intestine of freshly killed harp, hooded (*Cystophora cristata*), and bearded seals collected off northeast Newfoundland (see Brattey and Stenson 1993). Live adult nematodes, assigned to the genera *Contracaecum* or *Phocascaris* based on cephalic structures (Berland 1963; Fagerholm 1989), were washed twice in distilled water and stored frozen (−60°C) individually in labelled microcentrifuge tubes containing a drop of distilled water.

Electrophoretic methods

Whole larvae or small (3–4 mm) pieces of adult nematodes were homogenized in 15 μL of Tris–HCl (pH = 8) or distilled water and the homogenate was either used immediately or refrozen at −60°C. Initially, two types of electrophoresis were used: horizontal starch gels using methods described by Nascetti et al. (1986*b*) and cellulose acetate (Hebert and Beaton 1989). The following enzyme loci were screened: glucose phosphate isomerase (*Gpi*), Enzyme Commission (EC) number 5.3.1.9; glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*), EC 1.2.1.12; malate

Table 1. Details of the sources of larval nematodes collected from marine fishes off Newfoundland and Labrador.

Fish species	NAFO division	Collection dates	No. of samples examined[a]	No. of nematodes screened
Hippoglossoides platessoides	2J	July 1991	10	216
	2J	July 1992	24	112
	2J	August 1992	1	2
	3K	July 1991	2	24
	3L	April 1991	4	18
	3L	May 1991	1	9
	3L	April 1992	5	120
Gadus morhua	3L	July 1991	1	1
	3L	July 1992	4[b]	147
	3Ps	February 1991	3	8
	3Pn	February 1991	1	4
Mallotus villosus	3L	July 1991	4[b]	146
Myoxocephalus scorpius	3L	May 1991	1	32
Pleuronectes americanus	3L	May 1991	1	20

Note: Sampling locations are indicated on Fig. 1.

[a]Each sample consisted of nematodes recovered from 5–15 fish from a single set.

[b]Denotes samples which consisted of 50–100 fish collected either from cod traps (*G. morhua*) or from spawning beaches (*M. villosus*).

dehydrogenase (*Mdh-1*, *Mdh-2*, *Mdh-3*), EC 1.1.1.37; and phosphoglucomutase (*Pgm-1*, *Pgm-2*), EC 5.4.2.2. These enzymes are known to be useful for distinguishing the nematodes being investigated (Nascetti et al. 1986*a*, 1993; Orecchia et al. 1986*a*). Three buffer systems were used with the cellulose acetate gels: Tris–glycine (gel and electrode buffers 0.025 M Tris, 0.192 M glycine; pH = 8.5; run at 260 V for 30 min) for *Gpi*, and Tris-maleate (gel and electrode buffers 0.05 M Tris, 0.02 M maleic acid; pH = 7.8; 200 V for 35 min) or citric acid – *N*-(3-aminopropyl)-diethylamine (CAEA)(gel and electrode buffers 0.018 M citric acid, 0.030 M amine; pH = 8.5; 160 V for 40 min) for the remainder. Stain recipes were those given by Richardson et al. (1986) modified to give a total volume of 2 mL. An additional 2 mL of molten (60°C) agar (0.8%) was added to the staining solution just before applying to the gel. For additional details of electrophoretic methods see Hebert and Beaton (1989).

Loci were designated numerically reflecting decreasing electrophoretic mobility from the most anodal locus. Alleles were designated numerically according to their mobility relative to the most common allele (=*100*) in a reference population (*C. osculatum* A from a bearded seal from southern Labrador). Allelic frequencies were calculated from observed genotype numbers if all seven loci from 10 or more nematodes of a particular species were successfully screened. Some of the data for two polymorphic loci (*Pgm-1* and *Gpi*) were tested for significance of departures from Hardy–Weinberg equilibrium using the log-likelihood statistic, *G*, for goodness-of-fit (Sokal and Rohlf 1981). Rare alleles were pooled before testing and the test was not conducted if the observed frequency in any class was less than one; only four of the data sets were found to be sufficiently polymorphic to conduct these tests (see Results).

Initially, adult nematodes from seals were screened electrophoretically. Because a different support medium from that used by Nascetti et al. (1993) was used here, it was necessary to confirm that loci useful for distinguishing nematodes on starch gels were also useful when screened on cellulose acetate. Preliminary runs using the same samples on both types of gel confirmed that the two support media gave similar results. Adult nematodes from various seals were screened and identified, and homogenates prepared from adults of known genotype served as mobility controls on all gels. Preliminary runs using host tissues, and nematode samples frozen at −60°C for up to 3 year, indicated that nematodes were not contaminated with enzymes of host origin, and that freezing for extended periods did not adversely affect loci screened here. Although almost all gels were scorable, it was not possible to score all loci from each specimen; results from a minimum of three enzyme loci (two showing fixed differences among species plus a third exhibiting highly differentiated allelic frequencies) were used to identify nematodes to species. Several nematode specimens were damaged during removal from hosts and could not be used for the discriminant function analysis (DFA); however, these specimens were screened electrophoretically and identified to increase the sample sizes for genetic analyses.

Discriminant function analysis

Nematodes from Atlantic cod, capelin, and American plaice were used in the initial stages of the DFA. Seven measurements, chosen to capture the general body form of the larval nematodes (see below), were obtained from a total of 413 specimens. These were used as a training data set to select variables, select the most appropriate discrimination technique, and form a database to develop the DFs. The nematodes were immobilized by freezing them (−60°C) briefly (5 min) on a microscope slide, thawed, and straightened. Body length (LN) was measured by projecting an image of the specimen onto a sheet of paper and measuring the length with a curvimeter. Diameter of each nematode was measured at three locations: mid-length (DM), base of the caecum (DC), and at the anus (DA). Lengths of caecum (LC) and appendix (LA), and distance from the anus to the posterior tip of the tail (AP) were also recorded. These were measured to the nearest 10 μm using a compound microscope at ×40 or ×100 magnification equipped with an ocular micrometer. Subsequently, each nematode was homogenized and identified using biochemical markers as described above.

The performance of the DFs that were developed from the training data set were evaluated by cross-validation (Lachenbruch and Mickey 1968), where N - 1 of N training observations are used as a training data set to develop DFs that were applied to the one observation left out. This procedure was repeated for each of the observations.

A second group of larval nematodes, obtained from the same three species of fish, were measured and identified using electrophoresis as described above, except that only variables found to be useful for discriminating among the taxa were measured (see below). Specimens from this group were used as a test data set and classified to provide an independent assessment of the performance of the DFs developed from the training data set.

A third group of larval nematodes obtained from shorthorn sculpin (*Myoxocephalus scorpius*) and winter flounder (*Pleuronectes americanus*) was also collected, measured, identified, and classified as described for the test data set. These data were used to evaluate the performance of the DFs at classifying larval nematodes from fish species other than those on which the original DFs were based.

During classification by DFs it was assumed that the prior probability of a specimen belonging to each nematode species was equal. However, the proportions of each species in a sample could vary widely. To examine the effect of assuming equal prior probabilities, several subsets of the test data set containing different proportions of *C. osculatum* A, *C. osculatum* B, and *Phocascaris* sp. were constructed and classified using the DFs developed from the training data set. Subsets of sample size 100 were created by randomly selecting, with replacement, from the specimens in the test data set. These subsets were classified using the DFs and the resulting proportions compared with the known proportions obtained using biochemical markers. For each combination of proportions this procedure was repeated 100 times. A wide range of proportions was chosen to encompass most of the combinations likely to occur in nature.

Initially, the data were examined to determine whether the assumptions of homoscedasticity and multivariate normality for DFA were met. The Shapiro–Wilk test (Shapiro and Wilk 1965) revealed significant ($P < 0.05$) deviations from normality in 12 of the 49 data sets. However, Lachenbruch (1975) has shown that DFA is a robust technique that can tolerate some deviation from this assumption. The F_{MAX} test revealed significant heteroscedasticity ($P < 0.05$) among species in 6 of the 21 comparisons. The effect of deviating from this assumption was tested by comparing the results of DFA on the raw data with that conducted on log-transformed data (Pimentel 1979). These procedures gave nearly identical results, suggesting that the effect of deviating from this assumption was minimal. Pimentel (1979) also concluded that evidence consistently supported the robustness of the method and the effect of these deviations from the underlying assumptions was assumed to be minor.

Stepwise (forward) discriminant analysis (STEPDISC procedure in version 6.03 of the Statistical Analysis System, SAS 1988) was used as an aid to help select from the training data set variables that contributed most to the model for discriminating among the three nematode species. Of the seven variables, LC contributed little to the model ($F = 2.2$, $P = 0.1174$) and was omitted. Among the remainder, three contributed most to discriminating among species: LN, DM, and DC ($F = 339.6$, 101.9, and 34.7, all $P < 0.0001$). The value of Wilks' lambda with these three variables included was low (0.215), indicating that the model had reasonably good discriminatory power. Although variables LA, DA, and AP also had significant F values ($F = 9.5$, $P < 0.0001$; $F = 8.9$, $P < 0.0002$; $F = 4.5$, $P < 0.0119$), the corresponding partial R^2 values were low (≤0.045), and inclusion of these variables resulted in only minimal reductions in the value of Wilks' lambda (from 0.215 to 0.192), indicating that they contributed little to the discriminatory power of the model. Thus, the best variables for discriminating among the larvae of the three nematode species were LN, DM, and DC.

The DFs used for classification of the larval nematodes were based on a measure of generalized squared distance (Rao 1973). Initially, tests of the homogeneity of the within covariance matrix were conducted and the tests (one for data from each species of fish) were significant ($P < 0.0001$). Consequently, within-group covariance matrices for each nematode species were used for classification (Morrison 1976), yielding quadratic DFs. The training data sets were used to derive separate DFs for classifying nematodes from each species of fish. Coefficients for these DFs were stored and used to classify specimens from other data sets. Each specimen (x) from nematode species t can be classified by measuring the three variables selected by the stepwise procedure (i.e., LN, DM, and DC) and incorporating the values into the following quadratic DF:

$$[1]\quad D^2_t(x) = [a + (b\cdot\mathrm{LN}) + (c + \mathrm{LN}^2) + (d\cdot\mathrm{DM}) + (e + \mathrm{DM}^2) + (f\cdot\mathrm{DC}) + (g + \mathrm{DC}^2) + (h\cdot(\mathrm{LN}\cdot\mathrm{DM})) + \ldots + (i\cdot(\mathrm{LN}\cdot\mathrm{DC})) + (j\cdot(\mathrm{DC}\cdot\mathrm{DM}))]$$

where $D^2_t(x)$ is the generalized squared distance, LN is the nematode length, DM is the diameter at the midpoint, DC is the diameter at the base of the caecum, and a–i are coefficients for each nematode species generated from training

Table 2. Allele frequencies at seven enzyme loci in larval *Contracaecum osculatum* A, *C. osculatum* B, and *Phocascaris* sp. from Atlantic cod (*Gadus morhua*), American plaice (*Hippoglossoides platessoides*), and capelin (*Mallotus villosus*) collected off Newfoundland and Labrador.

		C. osculatum A	*C. osculatum* B			*Phocascaris* sp.		
Locus	Allele	American plaice	Atlantic cod	American plaice	Capelin	Atlantic cod	American plaice	Capelin
Mdh-1	*118*	0	0.009	0	0	0	0	0
	112	0	0	0	0	1.000	1.000	1.000
	110	0	0.004	0	0	0	0	0
	100	1.000	0.987	1.000	1.000	0	0	0
	N[a]	52	116	237	70	102	27	102
Mdh-2	*100*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	N	52	116	237	70	102	27	102
Mdh-3	*100*	1.000	0.957	0.983	1.000	0	0	0
	95	0	0.043	0.017	0	0	0	0
	92	0	0	0	0	1.000	1.000	1.000
	N	53	116	237	71	73	27	101
G3pdh	*100*	1.000	0	0	0	1.000	1.000	1.000
	90	0	1.000	1.000	1.000	0	0	0
	N	53	130	238	72	65	21	102
Pgm-1	*115*	0	0.014	0.028	0.038	0.688	0.778	0.676
	110	0.058	0.976	0.972	0.953	0.303	0.222	0.324
	100	0.933	0.010	0	0.009	0.008	0	0
	95	0.010	0	0	0	0	0	0
	N	52	104	232	53	61	27	54
Pgm-2	*105*	0	0	0.004	0.028	0	0.019	0.010
	100	0.942	0.928	0.907	0.887	0.977	0.981	0.990
	95	0.058	0.072	0.089	0.085	0.023	0	0
	N	52	104	231	53	64	27	51
Gpi	*100*	0.675	0.035	0.033	0.068	0.993	0.857	0.991
	90	0.275	0.942	0.967	0.898	0.007	0.143	0.009
	85	0.050	0.022	0	0.034	0	0	0
	N	20	113	15	59	67	27	54

[a]*N*, number of specimens screened.

data sets. The posterior probability of a specimen x belonging to nematode species t is equal to

$$[2] \quad p(t|x) = \exp(D_t^2(x))/\sum\exp(D^2(x))$$

Values of D^2 and p were calculated for each nematode species and specimens were classified to the species giving the lowest value of D^2 or the highest value of p.

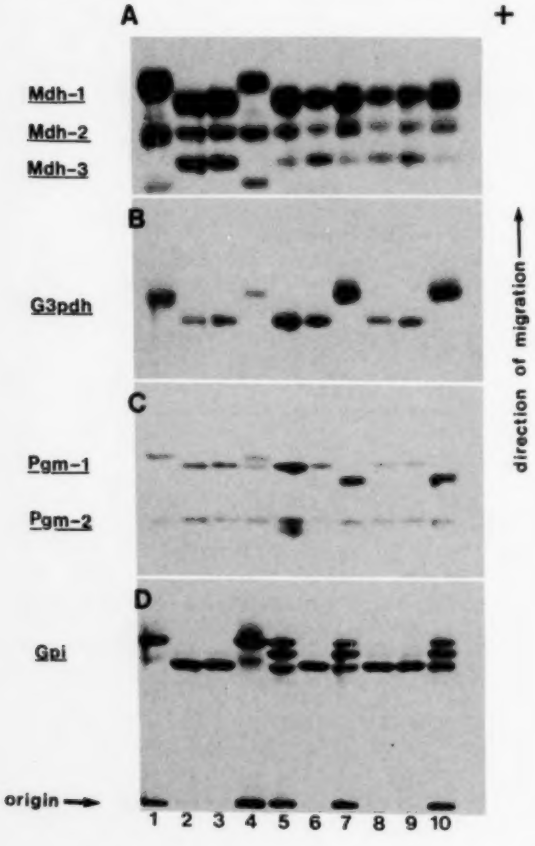
Results

Identification using allozymes

Electrophoresis revealed three genetically distinct groups that corresponded with adult nematodes previously reported from North Atlantic seals. Allelic frequencies at each locus for specimens from American plaice, Atlantic cod, and capelin are summarized in Table 2. These groups, which correspond to *C. osculatum* A, *C. osculatum* B, and *Phocascaris* sp. (see Nascetti et al. 1986*a*, 1993; Orecchia et al. 1986*a*; Brattey and Stenson 1993), are characterized by fixed genetic differences at three of the seven loci tested (*Mdh-1*, *Mdh-3*, *G3pdh*) and by highly differentiated allelic frequencies at another two loci (*Pgm-1*, *Gpi*). Each species is characterized by the following unique combination of allozymes in its genotype: *Phocascaris* sp.: $Mdh\text{-}1^{112}$, $Mdh\text{-}3^{92}$, and $G3pdh^{100}$; *C. osculatum* B: $Mdh\text{-}1^{100}$, $Mdh\text{-}3^{100}$, and $G3pdh^{90}$; *C. osculatum* A: $Mdh\text{-}1^{100}$, $Mdh\text{-}3^{100}$, and $G3pdh^{100}$. Typical electrophoretic patterns showing the most common alleles observed at each locus for adults and larvae of each species are shown in Fig. 2.

When the specimens were classified into the three groups using the combinations of fixed genetic differences described above, genotypic frequencies for the three polymorphic

Fig. 2. Zymograms illustrating the products of seven enzyme loci from nematodes of the *Contracaecum osculatum* s.l. – *Phocascaris* sp. complex from eastern Canada. (A) Malate dehydrogenase-1, -2, -3 (*Mdh*-1, *Mdh*-2, *Mdh*-3). (B) Glucose-3-phosphate dehydrogenase (*G3pdh*). (C) Phosphoglucomutase-1, -2 (*Pgm*-1, *Pgm*-2). (D) Glucose-phosphate isomerase (*Gpi*). Adults of *Phocascaris* sp., *C. osculatum* B, and *C. osculatum* A of known genotype were loaded into lanes 1, 6, and 10, respectively, and third-stage larvae were loaded into the remaining lanes. The zymograms for the adult nematodes correspond to the following genotypes: *Phocascaris* sp. (lane 1), $Mdh\text{-}1^{112/112}$, $Mdh\text{-}3^{92/92}$, $G3pdh^{100/100}$, $Pgm\text{-}1^{115/115}$, $Gpi^{100/100}$; *C. osculatum* B (lane 6), $Mdh\text{-}1^{100/100}$, $Mdh\text{-}3^{100/100}$, $G3pdh^{90/90}$, $Pgm\text{-}1^{110/110}$, $Gpi^{90/90}$; *C. osculatum* A (lane 10), $Mdh\text{-}1^{100/100}$, $Mdh\text{-}3^{100/100}$, $G3pdh^{100/100}$, $Pgm\text{-}1^{100/100}$, $Gpi^{90/100}$. The larva in lane 4 is *Phocascaris* sp., the larva in lane 7 is *C. osculatum* A, and all remaining larvae (lanes 2, 3, 5, 8, and 9) are *C. osculatum* B. Allele $Pgm\text{-}1^{110}$ occurs in *Phocascaris* sp. and *C. osculatum* B but is more common in the latter; allele Gpi^{100} occurs in all three species but at different frequencies (see Table 2). Two loci (*Mdh-2* and *Pgm-2*) were not useful for distinguishing the three species.



loci (*Pgm-1*, *Pgm-2*, and *Gpi*) were found to be in Hardy–Weinberg equilibrium ($G = 1.68$, $P > 0.1$ for *Gpi* in *C. osculatum* A from American plaice; $G = 0.02$, $P > 0.5$ for *Pgm-2* in *C. osculatum* B from American plaice; $G = 1.11$, $P > 0.1$ and $G = 0.20$, $P > 0.5$ for *Pgm-1* in *Phocascaris* sp. from capelin and Atlantic cod, respectively). There was no evidence of F_1 hybrids or of recombinant or backcross individuals among any of the larvae screened, indicating that there were three distinct gene pools in the samples of larvae tested.

Of the nematodes that were used in the DFA, 8.7% corresponded with *C. osculatum* A, 74.2% with *C. osculatum* B, and the remainder (17.1%) with *Phocascaris* sp. However, the overall percentage of each nematode species differed markedly among the five fish species (Table 3). American plaice harboured all three nematodes and were the only fish infected with *C. osculatum* A. Atlantic cod, capelin, and shorthorn sculpin all harboured both *C. osculatum* B and *Phocascaris* sp. Larvae of *C. osculatum* B were more common in four of the fish species, but *Phocascaris* sp. were more numerous in capelin (66.4% of 146). Concurrent infections of *C. osculatum* B and *Phocascaris* sp. were found in capelin, Atlantic cod, and shorthorn sculpin. American plaice often harboured both species within *C. osculatum* and rarely larvae of all three species of nematode.

Discriminant function analysis

Means of seven morphometric characters from larval nematodes from American plaice, Atlantic cod, and capelin from all locations combined are summarized in Table 4. However, there were unequal numbers of nematodes per sample and often several samples were collected from each species of fish; therefore, the means of the sample means rather than the overall arithmetic means were used in the DFA. These values give a more preferable estimate of the population mean for these variables (Williams 1952). The data suggest differences in many of the morphometric characters between at least two of the three nematode species. Mean lengths were generally greater for *Phocascaris* sp. (22.44–28.32) compared with *C. osculatum* A (18.25) and *C. osculatum* B (13.60–16.51) and the longest specimens (>25.0 mm) could probably be accurately classified as *Phocascaris* sp. based on length alone. However, for shorter specimens the lengths and other measurements often overlapped among species and no single variable could be used to accurately classify them.

Initially, three sets of DFs (one for each species of fish) were developed from the data, the first to discriminate among all three nematode species from American plaice, and the second and third to discriminate between *C. osculatum* B and *Phocascaris* sp. from Atlantic cod and capelin. In American plaice, 90.0 and 83.6% of the *Phocascaris* sp. and *C. osculatum* B, respectively, were classified correctly (Table 5). The results were poorer for *C. osculatum* A (74.4%), but the percentage correctly classified was much greater than would be predicted by random assignment (33.3%). Most of the errors involved misclassification of *C. osculatum* A as *C. osculatum* B and vice versa. In Atlantic cod and capelin, correct classifications were obtained for almost all specimens of *C. osculatum* B (98.7

Table 3. Percentages of *Contracaecum osculatum* A, *C. osculatum* B, and *Phocascaris* sp. recovered from five marine fish species collected off Newfoundland and Labrador.

Fish species	NAFO division	N^a	*C. osculatum* A (%)	*C. osculatum* B (%)	*Phocascaris* sp. (%)
Hippoglossoides platessoides	2J	330	18.2	76.1	5.8
	3K	24	29.2	54.2	16.6
	3L	147	5.4	91.2	3.4
Gadus morhua	3L	148	0	90.5	9.5
	3Ps	8	0	50.0	50.0
	3Pn	4	0	100.0	0
Mallotus villosus	3L	146	0	33.6	66.4
Myoxocephalus scorpius	3L	32	0	87.5	12.5
Pleuronectes americanus	3L	20	0	100.0	0

[a] *N*, number of nematodes screened and identified using biochemical markers (allozymes).

and 100%) and *Phocascaris* sp. (87.5 and 95.6%). These results indicate that the DFs based on nematodes from plaice cannot reliably classify *C. osculatum* A; however, most specimens of *C. osculatum* B and *Phocascaris* sp. are correctly classified irrespective of fish host.

Classification results for specimens from the test data set were similar to those obtained for the training data set. In American plaice, 89.7% of *C. osculatum* B and 100% of *Phocascaris* sp. were classified correctly, and most of the errors involved misclassifications of *C. osculatum* A (Table 6). In Atlantic cod and capelin, >95% of the *C. osculatum* B and ≥90% of the *Phocascaris* sp. were classified correctly.

Since the results of the classification of nematodes from Atlantic cod and capelin were similar, training data sets from these two species of fish were pooled and new DFs constructed for classifying specimens from either fish species. Pooling increased the sample sizes on which the new DFs were based. The accuracy of classification was marginally improved, with 98.4% of 102 *C. osculatum* B and 100% of 53 *Phocascaris* sp. classified correctly. When the pooled test data for these two fishes were used, good results were also obtained, with 98.9% of 89 *C. osculatum* and 96.8% of 68 *Phocascaris* sp. classified correctly.

The DFs based on the training data sets from Atlantic cod and capelin combined were also evaluated by classifying specimens from shorthorn sculpin and winter flounder. All 32 specimens from shorthorn sculpin and 20 from winter flounder were classified correctly, although the numbers of *Phocascaris* sp. in these fishes were low (7.7% of 52).

The DFs from the pooled data sets were also used to classify the structured test data sets containing various proportions of *C. osculatum* B and *Phocascaris* sp. In all cases classification resulted in proportions close to those known to occur in these randomly selected data sets (Table 7). Any bias owing to the assumption of equal prior probabilities on classification of unknown specimens from Atlantic cod or capelin is therefore probably small. However, the results do suggest a slight bias that increases from 1 to 4% in favour of *C. osculatum* B when the actual proportion of *Phocascaris* sp. in a sample increases from 50 to 100%.

The coefficients of the quadratic DFs, based on pooled specimens from Atlantic cod and capelin, are as follows:

[3] *C. osculatum* B: $D^2_1(x) = [-38.734 + (1.317 \cdot LN) + (-0.158 + LN^2) + (74.940 \cdot DM) + (-122.410 + DM^2) + (50.892 \cdot DC) + (-144.264 + DC^2) + (3.812 \cdot (LN \cdot DM)) + (1.232 \cdot (LN \cdot DC)) + (77.404 \cdot (DC \cdot DM))]$

[4] *Phocascaris* sp.: $D^2_1(x) = [-16.483 + (0.692 \cdot LN) + (-0.088 + LN^2) + (12.501 \cdot DM) + (-147.846 + DM^2) + (28.813 \cdot DC) + (-171.794 + DC^2) + (4.260 \cdot (LN \cdot DM)) + (0.872 \cdot (LN \cdot DC)) + (184.424 \cdot (DC \cdot DM))]$

Discussion

The recent discovery of sympatric sibling species among marine ascaridoid genera such as *Anisakis* (Nascetti et al. 1986*b*; Orecchia et al. 1986*b*), *Contracaecum* (Orecchia et al. 1986*a*; Nascetti et al. 1986*a*, 1993), and *Pseudoterranova* (Paggi et al. 1991) has created problems for biologists studying the larvae of these nematodes. Previously, the larvae could not be distinguished reliably and accurate information on even the most basic aspects of their biology was difficult to obtain. The present study attempts to mitigate these difficulties and shows that larvae of *C. osculatum* B and *Phocascaris* sp. are widely distributed and infect several fish species in waters around Newfoundland and Labrador. This finding is consistent with the broad geographic range and large number of seal species that can

Table 4. Means[a] (±SD) and ranges (mm) of seven morphometric characters from samples of third–stage larvae of the nematodes *Contracaecum osculatum* A, *C. osculatum* B, and *Phocascaris* sp. from American plaice (*Hippoglossoides platessoides*), Atlantic cod (*Gadus morhua*), and capelin (*Mallotus villosus*) collected off Newfoundland and Labrador.

		C. osculatum A		*C. osculatum* B		*Phocascaris* sp.	
Fish species	Character[b]	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
American plaice	LN	18.67±3.97	8.49–24.52	14.41±1.98	7.53–18.77	23.76±4.33	17.26–31.64
Atlantic cod	LN	—	—	16.15±2.13	11.64–18.36	28.60±3.42	23.56–32.47
Capelin	LN	—	—	14.69±1.67	11.92–17.81	23.16±4.25	13.29–32.74
American plaice	DM	0.69±0.10	0.45–0.86	0.66±0.07	0.47–0.83	0.70±0.10	0.50–0.87
Atlantic cod	DM	—	—	0.65±0.07	0.50–0.72	0.76±0.04	0.73–0.83
Capelin	DM	—	—	0.66±0.08	0.55–0.78	0.68±0.13	0.33–0.99
American plaice	DC	0.42±0.05	0.31–0.52	0.43±0.05	0.25–0.64	0.55±0.06	0.45–0.64
Atlantic cod	DC	—	—	0.41±0.04	0.36–0.50	0.53±0.07	0.46–0.64
Capelin	DC	—	—	0.43±0.06	0.31–0.55	0.50±0.07	0.31–0.65
American plaice	DA	0.15±0.03	0.11–0.23	0.14±0.03	0.07–0.32	0.17±0.06	0.12–0.21
Atlantic cod	DA	—	—	0.16±0.03	0.11–0.19	0.19±0.03	0.15–0.22
Capelin	DA	—	—	0.14±0.02	0.12–0.19	0.17±0.03	0.09–0.24
American plaice	LC	0.81±0.16	0.46–1.14	0.74±0.15	0.42–1.17	1.00±0.17	0.73–1.44
Atlantic cod	LC	—	—	1.04±0.20	0.72–1.36	1.12±0.13	0.95–1.23
Capelin	LC	—	—	0.82±0.17	0.53–1.06	1.07±0.20	0.57–1.88
American plaice	LA	1.27±0.21	0.57–1.64	1.12±0.18	0.61–1.53	1.37±0.25	0.86–1.84
Atlantic cod	LA	—	—	1.02±0.15	0.76–1.22	1.42±0.28	1.06–1.72
Capelin	LA	—	—	1.12±0.12	0.89–1.38	1.45±0.19	1.06–1.88
American plaice	AP	0.25±0.05	0.15–0.42	0.21±0.03	0.10–0.33	0.25±0.05	0.15–0.34
Atlantic cod	AP	—	—	0.21±0.03	0.15–0.24	0.28±0.04	0.25–0.34
Capelin	AP	—	—	0.23±0.04	0.17–0.32	0.27±0.05	0.17–0.37

[a]Numbers of specimens on which means in each row are based are 44, 260, and 20 for American plaice; 8 and 5 for Atlantic cod; 17 and 59 for capelin.

[b]LN, body length; DC, diameter at base of caecum; DM, diameter at midpoint; DA, diameter at anus; LC, length of caecum; LA, length of appendix; AP, length from anus to posterior tip.

serve as definitive hosts of these nematodes (Brattey and Ni 1992; Brattey and Stenson 1993). The higher relative abundance of larval *C. osculatum* B can probably be attributed to the huge population of heavily infected harp seals (Brattey and Ni 1992). Larvae of *Phocascaris* sp. were generally less numerous than those of *C. osculatum* B in fishes, which concurs with the much lower overall abundance of adult *Phocascaris* sp. in seals (Brattey and Ni 1992; Brattey and Stenson 1993). However, a notable finding was that larval *Phocascaris* sp. were more common than *C. osculatum* B in capelin, which contrasts with Pálsson and Beverley-Burton (1984), who surmised that most of the nematodes were *Contracaecum* sp. The restriction of *C. osculatum* A to flatfishes off Labrador is consistent with the more northerly distribution and benthic feeding habits of bearded seals, which are the only definitive hosts of this nematode in Canadian Atlantic waters (Brattey and Stenson 1993; Nascetti et al. 1993).

Discriminant function analysis revealed that there are differences in shape among third-stage larvae of the three nematodes, particularly between the two common species *C. osculatum* B and *Phocascaris* sp. Most larvae of *Phocascaris* sp. and *C. osculatum* B can be accurately identified by the DFs alone, whereas specimens of *C. osculatum* A are less reliably classified. Discriminant functions based on the three measurements used herein would therefore not be reliable for classifying larvae from areas or species of fish where *C. osculatum* A is found (i.e., American plaice off Labrador), which clearly limits their usefulness. However, larvae from hosts such as capelin, or from areas such as southern Newfoundland and possibly the Gulf of St. Lawrence where *C. osculatum* A is absent or extremely rare, can generally be assigned to the correct species using the DFs alone. The three measurements needed to evaluate the DFs are simple to obtain and could easily be employed for distinguishing larvae of *C. osculatum* B and *Phocascaris* sp. during surveys.

Although the DFs described herein were not completely successful at classifying larvae, the combined approach of DFA and identification using genetic markers could be

Table 5. Classification of larval nematodes *Contracaecum osculatum* A, *C. osculatum* B, and *Phocascaris* sp. using quadratic discriminant functions derived from morphometric data.

Fish species	Observed nematode species	Nematode species predicted by discriminant functions			
		C. osculatum A	*C. osculatum* B	*Phocascaris* sp.	Total
Hippoglossoides platessoides	*C. osculatum* A	29 (74.4)	7 (17.9)	3 (7.7)	39
	C. osculatum B	28 (14.4)	163 (83.6)	4 (2.1)	195
	Phocascaris sp.	1 (5.0)	1 (5.0)	18 (90.0)	20
Gadus morhua	*C. osculatum* B	—	76 (98.7)	1 (1.3)	77
	Phocascaris sp.	—	1 (12.5)	7 (87.5)	8
Mallotus villosus	*C. osculatum* B	—	25 (100.0)	0 (—)	25
	Phocascaris sp.	—	2 (4.4)	43 (95.6)	45

Note: Separate discriminant functions were used to classify specimens from each species of fish. Predicted results are compared with the correct classification obtained using biochemical markers. Equal prior probabilities were assumed during classification. Values in parentheses are percentages of row totals.

Table 6. Classification of an independant "test" sample of larval nematodes *Contracaecum osculatum* A, *C. osculatum* B, and *Phocascaris* sp. using quadratic discriminant functions derived from morphometric data. Separate discriminant functions were used to classify specimens from each species of fish.

Fish species	Observed nematode species	Nematode species predicted by discriminant functions			
		C. osculatum A	*C. osculatum* B	*Phocascaris* sp.	Total
Hippoglossoides platessoides	*C. osculatum* A	26 (72.2)	8 (22.2)	2 (5.6)	36
	C. osculatum B	21 (10.3)	182 (89.7)	0 (—)	203
	Phocascaris sp.	0 (—)	0 (—)	8 (100.0)	8
Gadus morhua	*C. osculatum* B	—	63 (96.9)	2 (3.1)	65
	Phocascaris sp.	—	1 (10.0)	9 (90.0)	10
Mallotus villosus	*C. osculatum* B	—	23 (95.8)	1 (4.2)	24
	Phocascaris sp.	—	0 (—)	52 (100.0)	52

Note: Predicted results are compared to the correct classification obtained using biochemical markers. Equal prior probabilities were assumed during classification. Values in parentheses are percentages of row totals.

used to develop keys for identifying larvae of other closely related nematode species. There are logistic difficulties associated with using these techniques on small nematodes but these can mostly be overcome. For example, larvae of nematodes often have few features that can be easily and reliably measured, and some potentially useful characters (such as position of the gland cell in the specimens examined here) did not meet these criteria and were omitted from the analyses. Also, the nematodes are prone to changes in dimensions because of muscle contraction, which may add variability to the measurements. By briefly freezing and thawing the specimens, the effects of muscle contraction were eliminated and the "posture" of the nematodes standardized. The effect of storage of live specimens in saline for long periods (i.e., >3 weeks) should also be investigated, because the dimensions of the nematodes may alter as energy reserves are depleted.

The possibility that DFs could be developed for classifying preserved specimens was not explored in the present study and would be feasible only if preliminary analysis showed that DFs developed from biochemically identified specimens were 100% accurate. Known specimens could then be fixed and remeasured and new DFs developed based on measurements from fixed material. This approach would greatly increase the usefulness of the DFs because freezing specimens could be avoided and archived collections of nematodes could possibly be identified, provided the specimens were preserved in a standard manner.

Table 7. Classification of samples of 100 nematodes containing different numbers of *Contracaecum osculatum* B and *Phocascaris* sp. using a quadratic discriminant function based on morphometric data.

Actual numbers		Predicted numbers		
C. osculatum B	*Phocascaris* sp.	*C. osculatum* B	*Phocascaris* sp.	Error rate
100	0	98.8	2.2	0.022
90	10	89.3	10.7	0.007
80	20	79.7	20.3	0.003
70	30	70.2	29.8	0.002
60	40	60.5	39.5	0.005
50	50	51.2	48.8	0.012
40	60	41.4	58.6	0.014
30	70	32.0	68.0	0.020
20	80	22.3	77.7	0.023
10	90	13.3	86.7	0.033
0	100	4.2	96.8	0.042

Note: Equal prior probabilities were assumed during classification. Predicted numbers in each row are the mean numbers of each species based on classification of 100 different random samples of 100 specimens (all standard deviations around the predicted means were <2).

One disadvantage of identification based on a DFA alone is that unknown nematode specimens must genuinely belong to one of the species from which the DFs are developed. If another species existed it could be erroneously classified to one of the known species. This possibility was minimized by collecting and screening large numbers of specimens from several species of fish collected over a wide area. The DFs described here should not be used to identify larvae from areas where other species may exist. Similarly, in projects where the costs of misclassifying a specimen are high, a threshold of posterior probability (equation 2) can be set such that, for example, specimens are only classified to a particular species if the posterior probability is >0.9. While this procedure may result in some specimens being classified as unknown, it can be used to minimize the possibility of erroneous classifications.

This study also shows that allozyme electrophoresis using a cellulose acetate support medium is an excellent technique for identifying individual larvae of marine ascaridoids such as *C. osculatum* s.l. and *Phocascaris* sp. The apparatus used is particularly suited to small animals that yield only minimal amounts (i.e., 10 μL) of homogenate. Furthermore, results are obtained more rapidly compared to starch gel electrophoresis, which requires run times of several hours versus only 25–45 min on cellulose acetate. Costs are also lower because smaller amounts of stain ingredients are required. More sophisticated techniques involving extraction and analyses of DNA could also be used to identify the larval nematodes (Curran et al. 1985; Sugane et al. 1989; Adamson et al. 1992), but these methods are slower and much more expensive. The electrophoretic technique used herein could also be useful from a medical perspective for identifying larvae of marine ascaridoids such as *Anisakis* and *Pseudoterranova* that can infect man. Although these genera were recently found to be comprised of several species (Nascetti et al. 1986*b*; Orecchia et al. 1986*b*; Paggi et al. 1991), it is not known which species are most pathogenic to man. Specimens removed from patients, or even fragments of specimens, could easily be identified to species using allozyme electrophoresis, thereby providing valuable information on the epidemiology of infection.

Acknowledgements

I thank Dr. D.J. Innes, Biology Department, Memorial University, St. John's, Nfld., and Professor L. Paggi and co-workers, Institute of Parasitology, University of Rome "La Sapienza," Rome, for advice on electrophoretic methods; N. Cadigan for programming advice; M. Brennan, P. Beck, K. Clark, E. Murphy, and V. Mercer for technical assistance; and D. Methven, Ocean Sciences Centre, St. John's; Dr. M. Hammill, DFO, Mont-Joli, Que.; and the staff of the Marine Mammals Section, NAFC, for help in collecting samples. Drs. D.G. Reddin and W.G. Warren kindly provided statistical advice and helpful comments on an earlier draft of the manuscript.

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Development of a diagnostic molecular marker for *Philonema* spp. (Nematoda; Dracunculoidea) infecting salmonids in British Columbia

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Abstract: We developed a species specific DNA probe based on differential PCR amplification that distinguishes two congeneric nematode parasites of salmonids in British Columbia: *Philonema agubernaculum* Simon and Simon, 1936, usually parasitic in lake resident rainbow trout, *Oncorhynchus mykiss*; and *P. oncorhynchi* Kuitunen-Ekbaum, 1933, parasitic in anadromous sockeye salmon, *O. nerka*. The region differentially amplified was the D3 expansion domain of the 28S rDNA. Sequences of the two species differ in two parts of the domain, one a single base substitution and the other a three base duplication in *P. oncorhynchi*. A primer specific to *P. oncorhynchi* (amplifying *P. oncorhynchi*, not *P. agubernaculum*) was defined in the duplication region. Using differential amplification, we showed that sockeye smolts are infected with *P. agubernaculum*, although returning adults harbour only *P. oncorhynchi*. This technique could conceivably be used to quantify the frequency of heterologous infections in the wild, before infecting worms are identifiable at the species level based on morphological criteria.

Résumé : Nous avons developpé un test diagnostique, basé sur l'amplification différentielle par PCR, qui permet de distinguer deux nématodes congénériques parasites de Salmonidés en Colombie-Britannique : *Philonema agubernaculum*, parasite des truites arc-en-ciel (*Oncorhynchus mykiss*) résidentes dans les lacs, et *P. oncorhynchi*, parasite du saumon rouge (*Oncorhynchus nerka*). La région amplifiée est le domaine D3 de l'ADN ribosomal 28S. Les séquences des deux espèces diffèrent en deux endroits : une substitution et une duplication de trois nucléotides chez *P. oncorhynchi*. Une amorce a été définie dans la région dupliquée de *P. oncorhynchi* et donc n'amplifie pas l'ADN de *P. agubernaculum*. Par amplification différentielle, nous avons montré que les jeunes saumons avant leur départ en mer étaient infestés par *P. agubernaculum*. Cette technique pourrait être utilisée pour quantifier la fréquence d"infestations hétérologues dans la nature, avant que les stades infestants ne soient morphologiquement différentiables.

Members of the genus *Philonema* are large (2–15 cm) worms in the body cavity of salmonid fishes in North America, Japan, and Siberian Russia. Two morphologically similar species of *Philonema* occur in salmonids in British Columbia, namely *P. oncorhynchi* Kuitunen-Ekbaum, 1933, originally described from sockeye salmon (*Oncorhynchus nerka*) caught in English Bay (Vancouver)(Kuitunen-Ekbaum, 1933) and *P. agubernaculum* Simon and Simon, 1936, originally described from rainbow trout (*O. mykiss*) in Wyoming (Simon and Simon 1936). Although original descriptions suggest that the two species can be distinguished morphologically, later studies cast doubt on this (Baylis 1948; Akhmerov 1955; Platzer 1964).

Adamson et al. (1992) recently showed that restriction fragment length polymorphisms (RFLP) could be used to distinguish the species. They showed that lake-resident *O. nerka*, or kokanee, harboured *P. oncorhynchi*. *Philonema agubernaculum* and *P. oncorhynchi* co-occur in British Columbia lakes such as Babine Lake, the former in *O. mykiss*, the latter in *O. nerka*. Because both species probably use the same copepod intermediate host it was postulated that parasite cross-infections might occur. In fact, seaward migrating sockeye salmon smolts from the Upper Fraser River drainage commonly contain larvae of *Philonema* although returning adults are free of these parasites. This

Received March 30, 1994. Accepted December 15, 1994. J12592

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Table 1. Summary of specimens of *Philonema* examined either by direct sequencing or by differential PCR amplification using primers specific for *Philonema oncorhynchi*.

Species	Host	Locality	Sequenced	Differential amplification
Philonema agubernaculum	*Oncorhynchus mykiss* (adults)	Okanagan Lake	2	4
	O. kisutsch (smolts)	O'Connor Lake	3	
P. oncorhynchi	*O. nerka* (adults)	Iliamna Lake, Alaska		2
		Kennedy Lake	3	3
		Great Central Lake	3	
		Tahltan Lake	3	
Philonema sp.	*O nerka* (smolts)	Stuart River	6	2

Note: Unless otherwise indicated, all localities are in British Columbia.

suggests that sockeye salmon smolts may acquire heterologous infections from resident rainbow trout in their nursery lakes.

The present study was undertaken (*i*) to sequence a region of DNA that could be used to distinguish between the two species of *Philonema* in British Columbia, (*ii*) to develop a rapid, easy to use diagnostic probe based on the polymerase chain reaction (PCR), and (*iii*) to use these markers to examine the possibility that sockeye salmon smolts are infected with *P. agubernaculum*.

Materials and methods

Specimens of *Philonema oncorhynchi* were collected from prespawning sockeye salmon, *Oncorhynchus nerka*, from Great Central Lake, Kennedy Lake, and Tahltan Lake on Vancouver Island, and from Iliamna Lake on Woody Island in Alaska. These salmon were returning adult fish that had been shown previously to harbour only *P. oncorhynchi* (Adamson et al. 1992). Fish from Vancouver Island were transported to the Pacific Biological Station, Nanaimo, B.C., where they were examined and the worms fixed and stored in 95% ethanol before being shipped to Vancouver for further processing. Fish from Alaska were examined at the site of capture; worms were fixed in 95% ethanol and delivered to Vancouver for analysis. Specimens of *P. agubernaculum* were collected from smolts of coho salmon, *O. kisutch*, that had been held in net pens in O'Connor Lake (Vancouver Island). Sockeye salmon, and consequently *P. oncorhynchi*, are absent from O'Connor Lake. Fish raised in net pens in O'Connor Lake acquire their parasites by feeding on copepods that have been infected with *P. agubernaculum* from resident rainbow trout, *O. mykiss* (Adamson et al. 1992). We also examined *P. agubernaculum* from *O. mykiss* from Okanagan Lake.

In addition, sockeye salmon smolts were collected from Stuart River of the upper Fraser River drainage system, fixed in 95% ethanol and transported to Vancouver for dissection. Two types of worms were found in these smolts: large worms were found in the body cavity of about 10% of fish dissected, whereas much smaller worms were found in the swimbladder of 80% of dissected fish.

Worms were individually homogenized in NTE (10 mM NaCl, 10 mM Tris, 1 mM EDTA, 1% SDS) and incubated 2 h at 65°C with Proteinase K (2 mg/mL), followed by phenol–chloroform extraction and alcohol precipitation, as previously described (Després et al. 1992).

The D3 domain in the 28S subunit was amplified using primers designed based upon the *Caenorhabditis elegans* 28S gene sequence (Ellis et al. 1986) and those of other organisms (Gutell et al. 1990): Ce-26S3A (5′-GACCCG-TCTTGAAACACGGA-3′, position 3304–3323) and Ce-26S3B (5′-TCGGAAGGAACCAGCTACTA-3′, position 4309–4328) described in Nunn (1992). Thirty amplification cycles (denaturation, 91°C, 30 s; annealing, 55°C, 1 min; polymerization, 72°C, 1 min) were carried out in a 100-μL final volume in 2.5 mM $MgCl_2$, using Taq polymerase (Promega) and the two primers present in equal concentration (0.5 mM). Double-stranded amplified DNA was purified on columns (Magic prep from Promega), then denatured for 5 min at 95°C with 1 mM of sequencing primer and used in dideoxy chain termination sequencing reactions (Sanger et al. 1977) using 35S-dATP and Sequenase 2.0 DNA Sequencing Kit (U.S. Biochemical Co.). The reaction mix, consisting of 2 μL sequenase reaction buffer, 2 μL labelling mix (diluted 10×), 1 μL DTT, 0.5 μL Mn buffer, 0.75 μL 35S-dATP, and 2 μL sequenase (diluted 8×), was added to tubes containing 1 μL sequencing primer and 9 μL purified PCR product and was held on ice for 2.5 min. Termination tubes containing 2.5 μL ddNTP received 3.8 μL of reaction mix and were allowed to stand at 40°C for 5 min before the reaction was stopped with 4 μL of stop solution. DNA sequences were unambiguously determined from both strands.

Material sequenced included three adult male and three adult female worms from sockeye salmon smolts from Stuart River; two females from rainbow trout from Okanagan Lake, three females from coho salmon from O'Connor Lake, and three worms from prespawning adult sockeye salmon from each of Kennedy Lake, Great Central Lake, and Tahltan Lake. Sequence alignments were performed using the program ESEE from Cabot (1987) and secondary structure figures generated using LoopViewer (Gilbert 1990). In addition, differential PCR amplification was conducted on three worms from prespawning adult sockeye salmon from Kennedy Lake, two worms from prespawning adult sockeye salmon from Iliamna Lake, Alaska, four worms from adult rainbow trout from

Fig. 1. Secondary structure of the D3 region of the 26S-like RNA of *Philonema agubernaculum*. The D3 region of *P. oncorhynchi* differs in the presence of a compensatory transition (U replaced by C) in loop I (asterisk) and in a GCU insertion in loop III (position outlined by rectangle).

Okanagan Lake, and two worms from sockeye salmon smolts from Stuart River (Table 1).

Results

The D3 domain of the *Philonema* material examined was 169–172 nucleotides (nc) long and similar to that of *C. elegans* (165 nc, Ellis et al. 1986) or of other eukaryotes (from 160 to 174 nc, Michot et al. 1990). Its secondary structure (Fig. 1) can be derived from the general eukaryote model proposed by Michot et al. (1990) and consists of a stem extending from the core region of the molecule 14 nc in length (labelled H14) to a loop containing five stem regions each with a terminal loop of variable size. The second and smallest of these stem–loop structures exhibits little variation leaving four variable stem–loops labelled I to IV, respectively. Two sequences were found, one common to worms from prespawning adult sockeye salmon (*P. oncorhynchi*), the other, representing *P. agubernaculum*. This latter sequence was common to large worms removed from sockeye salmon smolts from Stuart River, worms removed from coho salmon from net pens in O'Connor Lake, and worms from adult rainbow trout from Okanagan Lake. We were unable to collect or amplify DNA from small worms from the swimbladder of sockeye salmon smolts.

Sequences of *P. agubernaculum* differ from those of *P. oncorhynchi* in two regions: a transition in stem I corresponding to a compensatory change (retaining the secondary structure) and one three nt insertion in loop III. This insertion, CGU, corresponds to a motif repeated twice in *P. agubernaculum* and three times in *P. oncorhynchi*. None of these changes affects the stability of the secondary structure.

Fig. 2. Primers used in the present study in an attempt to differentially amplify the D3 regions of *Philonema agubernaculum* and *P. oncorhynchi*. PONCO preferentially amplified DNA of *P. oncorhynchi* : it had three internal mismatches to the *P. oncorhynchi* sequence and six mismatches, including the two 3′ bases, to that of *P. agubernaculum*.

PONCO	5′- ATGGGATCGTAGACTTAG-3′
Philonema agubernaculum	5′- ATGGGATCGTCGTAGTGA-3′
P. oncorhynchi	5′- ATGGGATCGTCGTCGTAG-3′

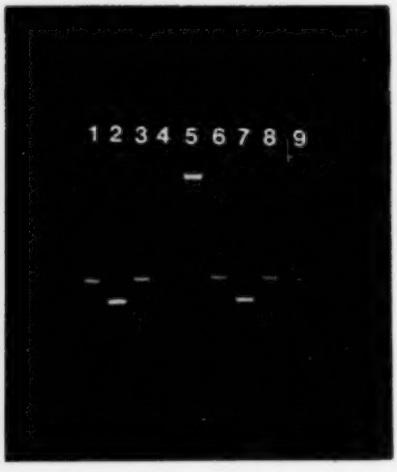
A search of the sequence differences using PC/Gene revealed no restriction enzyme that would cut differentially. We therefore designed primers to differentially amplify a subregion of the D3. We defined a primer (PONCO 5′-ATGGGATCGTAGACTTAG-3′) that included three internal mismatches with *P. oncorhynchi*, but with a perfect match in the three nucleotides at the 3′ end. This primer included six mismatches with *P. agubernaculum*, including the two terminal 3′ nucleotides (Fig. 2). Using PONCO and Ce-26S3B as amplification primers and an annealing temperature of 40°C (the PCR amplification program being otherwise as described above) we specifically amplified *P. oncorhynchi* and not *P. agubernaculum* (Fig. 3).

Discussion

The D3 sequence of *P. oncorhynchi* collected from prespawning sockeye salmon differed from that of *P. agubernaculum* from O'Connor Lake by a transition and an additional three nucleotide insertion. Specimens of *P. agubernaculum* from rainbow trout in Okanagan Lake gave sequence identical to that obtained from worms collected from O'Connor Lake. Two morphological types of *Philonema* were removed from seaward migrating sockeye salmon smolts. Large worms were found in the body cavity of about 10% of the fish; most of these were adult males and females, many of the females being larvigerous. Small worms were found in the lining of the swimbladder; these appeared to be third-stage larvae of *Philonema*. We were unable to amplify and sequence material from the larval worms, but sequences from the large worms were identical to that from *P. agubernaculum*.

This finding demonstrates that sockeye salmon smolts are infected by *P. agubernaculum* in nature and raises the question as to how hybridization is prevented between this species, which is normally a parasite of resident *O. mykiss*, and *P. oncorhynchi*, which typically occurs in *O. nerka*. Bashirullah (1966, 1983) stated that *P. oncorhynchi* develops slowly and depends on host hormonal cues for final maturation; thus, gravid worms are found only in upstream adult migrants. *Philonema agubernaculum*, on the other hand, develops directly to adulthood independent of host hormonal status and Bashirullah suggested they were unable to survive the long marine sojourn of *O. nerka*. Thus, *O. nerka* represents a dead end host for *P. agubernaculum* and by the time *P. oncorhynchi* reaches maturity, all *P. agubernaculum* will have perished. This hypothesis is consistent with the present findings.

Fig. 3. Differential polymerase chain reaction amplification of *Philonema* from west coast salmonids. Source of material by lane from left to right. Lane 1, *P. oncorhynchi* from prespawning adult sockeye salmon (*O. nerka*) from Iliamna Lake, Alaska, amplified using D3 primers Ce–26S3A and Ce–26S3B. Lane 2, same as lane 1 but using primers PONCO and Ce–26S3B. Lane 3, *Philonema* sp. from downstream migrating sockeye salmon smolts from the Stuart River, B.C., amplified using D3 primers, Ce–26S3A and Ce–26S3B. Lane 4, same as lane 3 but using primers PONCO and Ce–26S3B. Lane 5, 1-kb ladder (BRL). Lane 6, *P. oncorhynchi* from prespawning adult sockeye salmon from Kennedy Lake, Vancouver Island, amplified using D3 primers Ce–26S3A and Ce–26S3B. Lane 7, same as lane 6 but using primers PONCO and Ce–26S3B. Lane 8, adult *P. agubernaculum* from adult rainbow trout from Okanagan Lake, B.C., amplified using D3 primers Ce–26S3A and Ce–26S3B. Lane 9, same as lane 8 but using primers PONCO and Ce–26S3B.



Primers developed here differentially amplify the D3 region of *P. oncorhynchi* from one Alaskan and several Vancouver Island localities. We do not know whether the D3 region differs in worms from other regions (e.g., Fraser and Skeena River drainage areas). Adamson et al. (1992) found no fixed differences in restriction fragment banding patterns among worms from sockeye salmon collected from the lower Fraser River, Vancouver Island, or Skeena River drainage systems. It is possible that gene flow has been sufficient to maintain this region constant. Unlike sequences of single copy genes, ribosomal sequences are thought to exhibit concerted evolution that acts to prevent rapid interpopulational divergence as long as some gene flow exists between populations (Dover 1989). Thus, the PONCO primer may successfully amplify all populations of *P. oncorhynchi*.

Clearly, failure of PONCO to amplify does not mean the species in question is *P. agubernaculum*; it means only that it is likely not *P. oncorhynchi* . Populations of *P. agubernaculum* probably display more variation in the D3 and other regions. This species has been reported from resident salmonids of various genera (*Coregonus*, *Prosopium*, *Salvelinus*, *Salmo*, *Oncorhynchus*) in western and eastern North America (Chacko 1975; Margolis and Arthur 1979). Some of these populations may have been separated from each other for many millions of years and their DNA may have diverged considerably from one another. Nevertheless, D3 sequences of material examined from Vancouver Island and the Stuart River were identical.

Speciation in *Philonema* can be conceived as having occurred on two planes. Vicariant forms occur in resident salmonids from different geographical regions. For example, *P. salvelini* Richardson, 1937 in *Salvelinus fontinalis* in Quebec, *P. agubernaculum* in salmonids in western North America, and *P. sibirica* Bauer, 1946 in *Coregonus* spp. in Russia. Note that these are nominal species and should be compared genetically. Vicariance may also have given rise to the various species in anadromous *O. masou* in Japan, *O. nerka* in North America, and *Salvelinus alpinus* in Greenland. The other form of speciation concerns the derivation of forms adapted to anadromous hosts from those occurring in non-anadromous hosts (and (or) vice versa). This involves adaptive coevolution.

Adamson et al. (1992) proposed that *P. oncorhynchi* developed in *O. nerka* as a capture (speciation involving a host switch) from some non-anadromous fish host. It is presumably not the anadromous quality that forced speciation, since *P. oncorhynchi* occurs in non-anadromous *O. nerka* (kokanee) where these occur sympatrically with anadromous forms (Adamson et al. 1992). Furthermore, there is no evidence of physiological incompatibility per se, because *P. agubernaculum* is able to survive, mate, and even produce first-stage larvae in *O. nerka*. Rather, the incompatibility appears to derive from inappropriate developmental timing. Thus, *P. agubernaculum* may not be able to use anadromous *Oncorhynchus* as hosts because its development is not synchronized with their 4- to 5-year spawning cycle.

Acknowledgments

Stewart Johnson and Reginald Blaylock (D.F.O., Pacific Biological Station, Nanaimo) helped with collection of Vancouver Island samples. Chris Bull (B.C. Environment, Fisheries Management Program, Penticton) supplied samples from Okanagan Lake, Colin Levings (D.F.O., West Vancouver Laboratory) supervised collection of smolts from Stuart River, and Chris Foote (College of Fisheries, University of Washington, Seattle) supplied samples from Alaska. Daniel Yeulett and Martin Rebele helped with extraction of DNA and PCR. This study was supported in part by a grant from NSERC (UBC-80875) to M.L.A. and a Lavoisier Fellowship from the French Ministry of Foreign Affairs to L.D.

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Development to third-stage larvae occurs in the eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae)

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Abstract: Two moults occur during larval development in the eggs of *Anisakis simplex* (Rudolphi, 1809) and *Pseudoterranova decipiens* (Krabbe, 1878) from the North Atlantic. Live larvae forced out of eggs in sea water by coverslip pressure shortly before spontaneous hatching were surrounded by the thin cuticle of the first-stage larva. Infective larvae from naturally hatched eggs are loosely ensheathed in the thick cuticle of the second-stage larva. Thus, it is the third-stage larva that emerges from the egg of both species and not the second-stage larva as previously believed. The thin, smooth, fragile cuticle of the first-stage larva remains in the egg. The striated, cocoon-like cuticle of the second-stage larva of *A. simplex* may increase the buoyancy of the third-stage larva. The tail tip of the cuticle of the second-stage larva of *P. decipiens* is sticky and adheres the sheathed third-stage larva to the substrate.

Résumé : Les larves d'*Anisakis simplex* (Rudolphi, 1809) et de *Pseudoterranova decipiens* (Krabbe, 1878), espèces de l'Atlantique Nord, subissent deux mues durant leur phase de développement dans l'oeuf. Des larves vivantes qu'on a extraites de l'oeuf dans l'eau salée à l'aide d'une lamelle avant l'éclosion spontanée étaient entourées de la mince cuticule des larves de premier stade. Les larves infectantes issues d'oeufs éclos naturellement sont lâchement engainées dans l'épaisse cuticule des larves de deuxième stade. Chez ces deux espèces, ce sont donc des larves de troisième stade qui émergent de l'oeuf, et non pas des larves de deuxième stade, comme on le pensait antérieurement. La cuticule de la larve de premier stade, mince, lisse et fragile, demeure dans l'oeuf. Chez *A. simplex*, la cuticule de la larve de deuxième stade, striée et en forme de cocon, pourrait accroître la flottabilité de la larve de troisième stade. L'extrémité terminale de la cuticule de la larve de deuxième stade de *P. decipiens* est collante et retient la larve de troisième stade au substrat.

[Traduit par la Rédaction]

Introduction

Encapsulated larvae of the nematodes *Anisakis simplex* (Rudolphi, 1809) and *Pseudoterranova decipiens* (Krabbe, 1878) are common on the viscera and in the musculature of many marine teleosts from the North Atlantic (see, e.g., Berland 1961; McClelland et al. 1990; Køie 1993*b*).

Received January 18, 1994. Accepted December 15, 1994. J12590

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These larvae, each with a characteristic "boring tooth," are all considered third stage (e.g., by Berland 1961; Smith and Wootton 1984*a*, 1984*b*; Likely and Burt 1989). In the definitive host two moults take place to produce the fifth stage, which grows to maturity. Larvae of both species are believed to moult once within the egg (see, e.g., Scott 1955; Oshima et al. 1969; Grabda 1976; Smith and Wootten 1978; Smith 1983*b*; Burt et al. 1990; Brattey and Clark 1992). The eggs of both species should consequently contain the second-stage larva surrounded by the shed cuticle of the first-stage larva.

If the above view is correct, the second moult might occur in the haemocoel or body cavity of an invertebrate or fish host. Such a moulted cuticle surrounding the "larva with boring tooth" of *A. simplex* and *P. decipiens* has never been observed with certainty. In this communication we

study the larval development in the eggs of *A. simplex* sensu lato and *P. decipiens* sensu lato to determine whether the larvae leaving the eggs are in the second or third stage.

Recent genetic studies on the taxonomy of *A. simplex* from the Mediterranean and the Northeast Atlantic have shown that at least two sibling species, provisionally named *A. simplex* A and *A. simplex* B by Nascetti et al. (1986), exist. The geographic distributions of *A. simplex* A and *A. simplex* B appear to be mainly Mediterranean for the former and mainly North Atlantic for the latter. Since electrophoretic work was not done, it is not possible to assign *A. simplex* in the present study to *A. simplex* A or B with certainty. However, they are likely *A. simplex* B.

Electrophoretic work has shown that in the North Atlantic and adjacent seas, *P. decipiens* is a complex of at least three sibling species: *P. decipiens* A, B, and C (Paggi et al. 1991). In Canadian Atlantic waters, where *P. decipiens* A is apparently absent, *P. decipiens* B infects both grey seal (*Halichoerus grypus*) and harbour seal (*Phoca vitulina*). *Pseudoterranova decipiens* C has only been recorded in the bearded seal. Therefore, *P. decipiens* in the present study is likely *P. decipiens* B. In addition to the sibling species found in both grey and harbour seals, a different type has also been found in the grey seal (but not in the harbour seal), as reported by Burt (1994).

Materials and methods

Live specimens of *Anisakis simplex* sensu lato were obtained from the stomach of an adult male harbour porpoise (*Phocoena phocoena*) caught and drowned in a gill net off Bergen, Norway, on 5 June 1993. At the Marine Biological Laboratory in Helsingør, eggs were collected from gravid females using a method developed by D.P. Højgaard (FR-660 Søldarfjordur, Faroe Islands, unpublished data). The eggs were kept in sea water (30 o/oo) in Petri dishes at 6°C. Larvae in various stages of development were forced from live eggs in sea water by coverslip pressure. Some eggs were also allowed to hatch spontaneously.

Specimens of *Pseudoterranova decipiens* sensu lato were obtained from a harbour seal caught in Passamaquoddy Bay, New Brunswick, Canada, on 12 March 1994. In Helsingør the eggs were removed from the worms and transferred to sea water in Petri dishes at 20°C. The eggs and larvae were treated as those of *A. simplex*. Measurements of larvae are based on live specimens.

Results

Results are shown in Figs. 1 and 2. Two moults were observed to take place in the eggs of both species, one occurring shortly after the other. Since the mature specimens of *A. simplex* and *P. decipiens* were already about 1 and 2 weeks old, respectively, when the observations were started, the exact time for each ecdysis is impossible to determine. However, when observations started, most eggs of both species were between the four-cell stage and the morula stage. Eggs of *A. simplex* hatched between 3 and 4 weeks post-incubation at 6°C, whereas those of *P. decipiens* (Fig. 2A) hatched within 8 d at 20°C. For *A. simplex* the first ecdysis (Figs. 1 A–1C) was observed about 2 weeks post-incubation, the second ecdysis occurring 3–4 d later. For *P. decipiens*, the first ecdysis started at 4–6 d post-incubation and the second ecdysis started 2–3 d later. Because of the mechanical treatment of the eggs the thin, smooth, fragile cuticle of the first-stage larva was most often broken (Figs. 1A, 1B, 2B, and 2D), but in a few cases large parts of the cuticle were seen (Figs. 1C and 2C). In naturally hatched eggs, the cuticle of the first-stage larva remains in the egg. The thick, rough cuticle of the second-stage larva starts to detach apically and distally (Figs. 1D and 2E). In the beginning, the cuticle is tight fitting (Figs. 1E and 2F). Movement by the larvae causes the cuticle to stretch and shortly before natural hatching the cuticle becomes more loose fitting (Figs. 1D, 1F, and 1G). The cuticular lining of the excretory duct forms a long, thin string shortly after the ecdysis (Figs. 1D and 2F). The excretory duct of older larvae is collapsed and closes the excretory pore. The cuticular lining of the buccal cavity (Fig. 2F) appears in older larvae as a crumpled mass (Figs. 1D and 1F). Fully developed larvae and larvae from spontaneously hatched eggs are closed apically by a small knob (Figs. 1G and 2G).

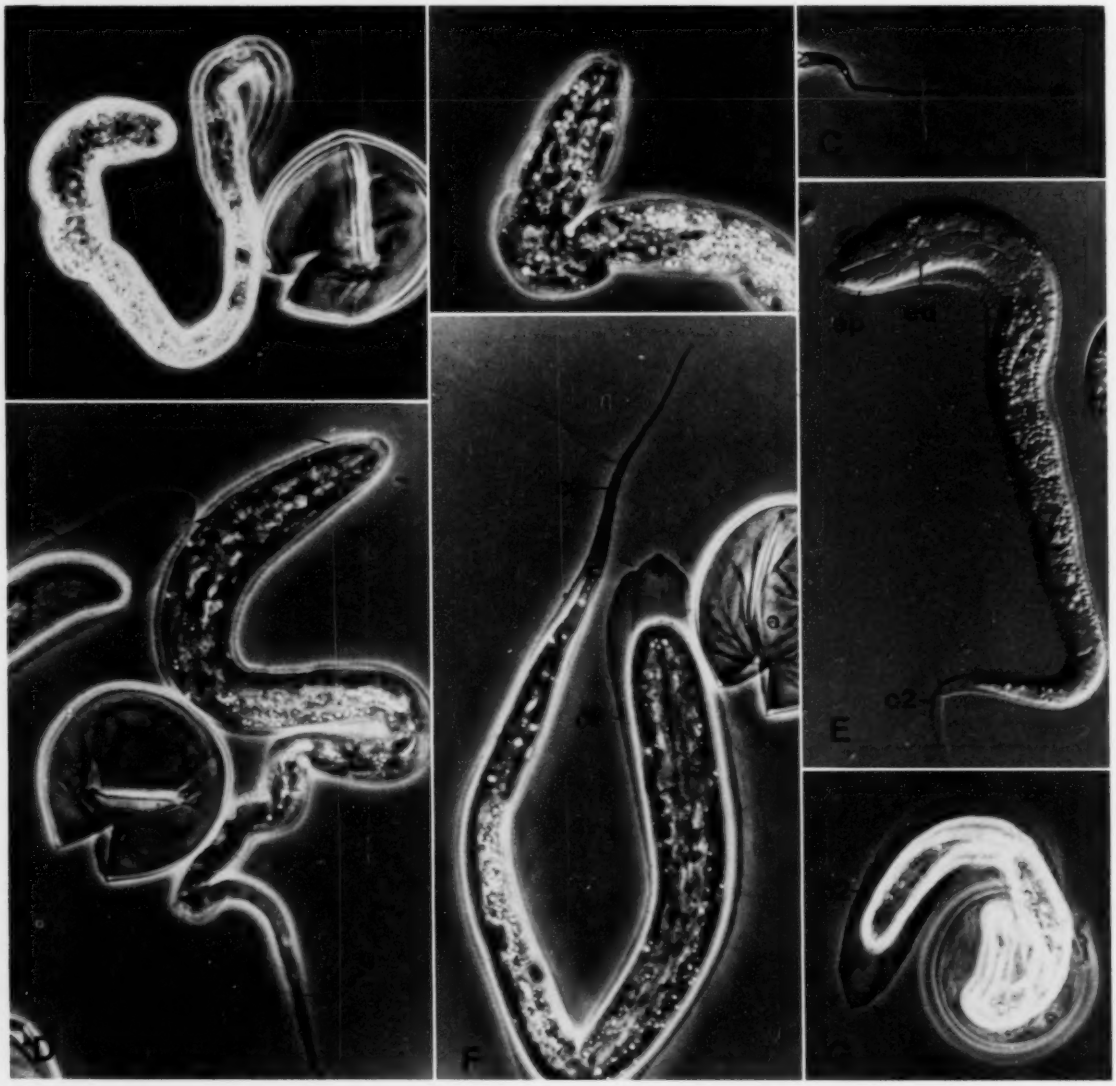
The tail tip of the first-stage and second-stage larvae of both species is long, pointed, and without refractile granules (Figs. 1C and 2D). The blunt tail of the third-stage larvae is surrounded by the long, pointed cuticle of the second-stage larvae (Figs. 1E, 1F, 2F, and 2G). The larvae of both species are unable to free themselves from the cuticular sheaths.

Short lateral invaginations, seen apically in the second-stage cuticle of *A. simplex*, are interpreted as the cuticular lining of the lateral sense organs, the amphids (Figs. 1D, 1F, and 1G). Similar structures were not observed in the second-stage cuticle of *P. decipiens*. However, if present they would be difficult to detect, since ensheathed third-stage larvae when examined under a coverglass were usually seen laterally (Figs. 2F and 2G).

Larvae of *A. simplex*, 250–280 μm in length, emerging from spontaneously hatched eggs retained the cuticle of the previous second-stage larva as a loose-fitting, finely transverse striated sheath. The whole cuticle, and especially the two lateral flanges or fin-like folds, are elastic. When the third-stage larva rests on the bottom of the dish, the cuticle gradually becomes more tight fitting. Wriggling movements cause the cuticle to stretch and actively swimming larvae were always ensheathed within a loose-fitting cuticle. Whereas the eggs always remained on the bottom of the Petri dish, live larvae were always suspended in the water.

Larvae of *P. decipiens*, 200–220 μm in length, are ensheated in the thick cuticle of the second-stage larva. The infective larvae (Figs. 2G and 2H) are surrounded by a cocoon-like sheath. No remains of rectum, buccal cavity, and excretory system of the second-stage larva were observed. No lateral flanges occur. The cuticle surrounding the pointed tail tip of the second-stage larva is sticky, and the third-stage larva adheres to the substrate by means of the loose cuticle only (Fig. 2G). The third-stage larva, which has a well-developed boring tooth and alimentary tract, is active and makes flicking movements by alternately extending and bending its body. It does not detach itself from the substrate. The larvae remained active at 20°C for at least 5 d and during this period no larvae died.

Fig. 1. Live larvae of *Anisakis simplex* forced out from eggs by coverslip pressure. (A) Early second-stage larva. The cuticle (*c1*) of the first-stage larva is loosely attached. (B) Young second-stage larva. The thin, smooth cuticle is interpreted as *c1*. The rough cuticle of the second-stage larva is seen below. (C) Tail tip of second-stage larva surrounded by *c1*. (D) Second-stage larva with detached *c2* anteriorly and the anterior end of a third-stage larva surrounded by the loose-fitting *c2*. Note lateral flanges, cuticular lining of the excretory duct (*ed*), position of excretory pore (*ep*), and, laterally, structures (arrowheads) believed to be the lining of the amphids of the second-stage larva. (E) Young third-stage larva ensheathed by *c2*. The boring tooth, oesophagus, excretory pore (*ep*), excretory duct (*ed*), and rectum are seen. (F) Fully developed third-stage larva loosely ensheathed by *c2*. Amphids (arrowheads) and excretory duct of the second stage larva are seen. (G) Third-stage larva shortly before spontaneous hatching. Arrowheads show the cuticular lining of the amphids. (A–D, F, and G) Phase contrast. (E) Interference contrast. All to same scale.

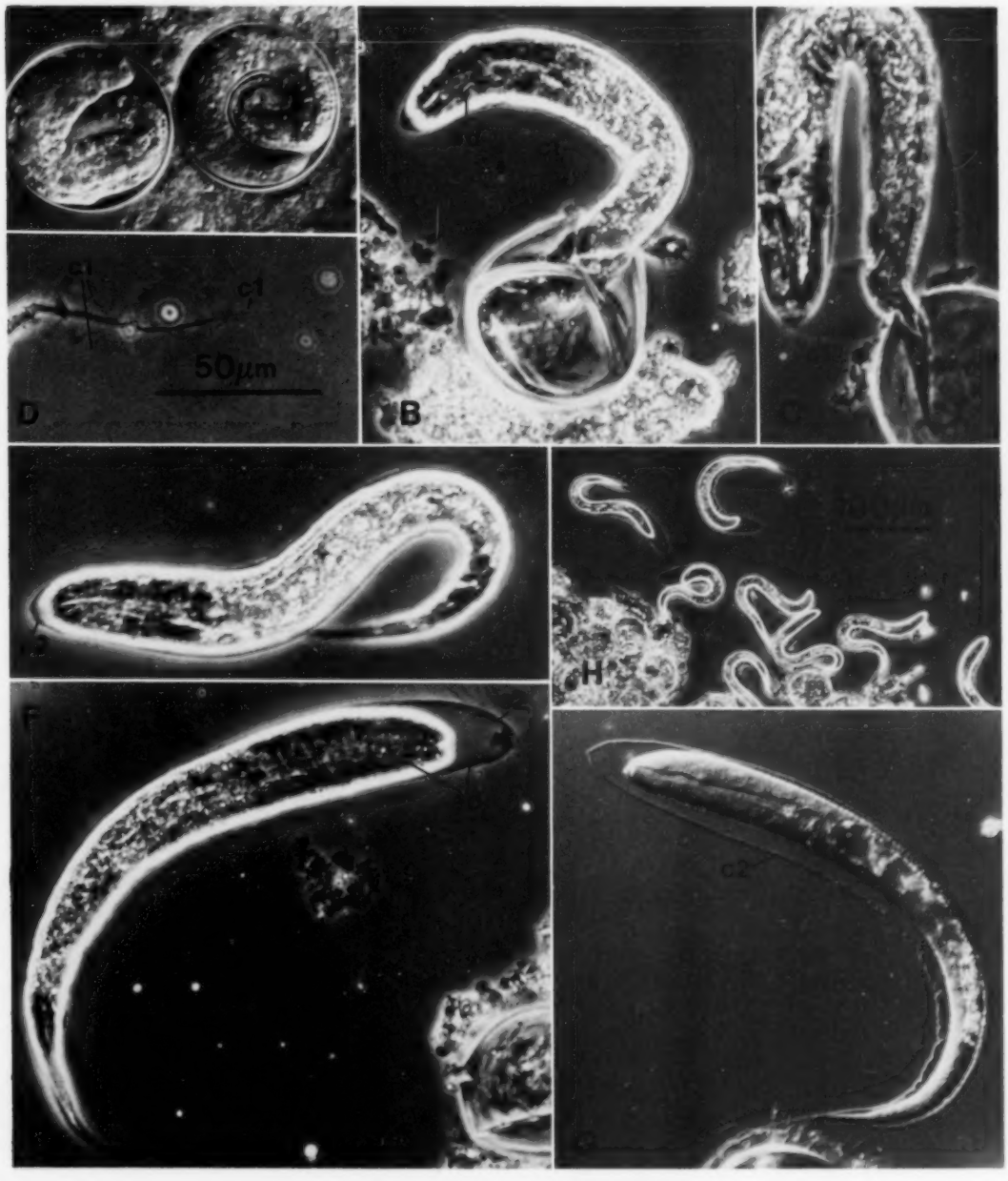


Discussion

It has recently been shown that two ecdyses take place within the eggs of the anisakids *Hysterothylacium aduncum* (Rudolphi, 1802) sensu lato and *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto and that these larvae therefore leave the eggs as third-stage larvae (Køie 1993*a*; Køie and Fagerholm 1993). The present study shows that *Anisakis simplex* and *Pseudoterranova decipiens* also emerge from the egg as third-stage larvae.

The hatching time of *A. simplex* at 6°C in the present study is broadly similar to those reported for this species by van Banning (1971) and Brattey and Clark (1982).

Fig. 2. Live eggs (A) and larvae of *Pseudoterranova decipiens* forced out from eggs (B–F) and naturally emerged (G and H). (A) Eggs a few days before spontaneous hatching. That to the left probably contains a first-stage larva, the other a second-stage larva. (B) Early second-stage larva partly surrounded by the cuticle of the first-stage larva (*c1*). Note the excretory duct (*ed*). (C) The thin, smooth cuticle of the first-stage larva is detached from the middle of the larva and is evaginated over the apical end and the posterior part of the larva. The rough cuticle of the second-stage larva is seen below. (D) Tail tip of second-stage larva surrounded by the detached cuticle of the first-stage larva. (E) Second-stage larva during ecdysis. The cuticle (*c2*) is detached apically. (F) Slightly older, now third-stage larva, with the cuticle of the second-stage larva detached apically and distally. Note the excretory duct of the third-stage larva (*ed*) and the cuticular linings of the excretory duct and buccal cavity of the second-stage larva. (G) Infective third-stage larva, with well-developed boring tooth, attached to an egg by the cocoon-like cuticle of the second-stage larva. (H) Infective larvae attached posteriorly to the substrate. (A and G) Interference contrast. (B–F and H) Phase contrast. A–G to same scale.



van Banning (1971) regarded the sheathed larva of *A. simplex* (syn.: *A. marina* Linnaeus, 1767) as the first-stage larva. Grabda (1976) described the sheath surrounding the larva from naturally hatched eggs as the moult of the first-stage larva. Brattey and Clark (1992) observed that the larva of *A. simplex* B retained the cuticular sheath, presumably from the first moult.

The present hatching time of *P. decipiens* agrees broadly with those observed by Scott (1955) and McClelland (1982). A longer hatching time is recorded when the eggs are kept at lower temperatures, similar to those encountered under natural conditions (McClelland and Ronald 1974*a*; McClelland 1982; Brattey 1990; Burt et al. 1990). However, whereas the larvae in the present study remained active for at least 5 d at 20°C, those studied by McClelland (1982) expired within 24–48 h at the same temperature. The length of the live larvae of *P. decipiens* from spontaneously hatched eggs in the present study is similar to that observed by McClelland (1982) for fresh heat-relaxed specimens. Larvae measured after fixation in 10% formalin are much shorter (Brattey 1990).

The present study and the studies by Køie (1993*a*) and Køie and Fagerholm (1993) do not indicate great morphological or functional differences between the sheaths (cuticle of second-stage larva) of the examined members of Anisakidae: *A. simplex*, *P. decipiens*, *C. osculatum*, and *H. aduncum*. The larvae of *A. simplex* and *C. osculatum*, which are able to swim and mainly use pelagic crustacean hosts, are surrounded by sheaths with lateral extensions, which may be an adaptation to pelagic conditions. The striations on the cuticle of the second-stage larva of *A. simplex* are less conspicuous than those of *C. osculatum*. Even though Smith (1983*a*) did not find great differences in the sedimentation rates for eggs and recently emerged larvae of *A. simplex*, it is obvious from the present observations that the cuticular sheath increases the buoyancy of the larvae. The benthic *P. decipiens* has a more tight-fitting cuticle without lateral flanges. The cuticular sheath is apparently closed, since the excretory duct is collapsed, and there are no openings anteriorly or remains of the rectum.

When ingested by crustaceans, the larvae of both species are probably released from the second-stage cuticle by the action of the mouthparts of the intermediate host. This allows the third-stage larvae to penetrate the host gut prior to establishing themselves in the haemocoel. Observations by A. Jarecka and M.D.B. Burt (unpublished data) indicate that when several crustaceans (e.g., *Artemia salina*, large *Gammarus oceanicus*) ingest the ensheathed larvae of *P. decipiens* the second-stage sheath is not ruptured and the still ensheathed larvae pass out of the gut with the faeces.

Valter et al. (1982) observed amphids in the third-stage larva of *A. simplex* removed from a fish host. The present study shows that the second-stage larva of *A. simplex* already has amphids. Amphids were described from cultured third-stage (believed second-stage) larva of *P. decipiens* by McClelland and Ronald (1974*a*), but they were not observed on SEM micrographs of the third-stage larva of *P. decipiens* from a fish host (Valter et al. 1982).

Whereas it has not been previously argued that the larva of *A. simplex* may develop to the third stage in the egg, McClelland (1990) proposed that *P. decipiens* may do so because neither moulting nematodes nor cast cuticles were found in experimentally infected crustaceans. Prehatch development to the third stage in the egg has been reported in other ascaridoid genera (see McClelland 1990; Køie and Fagerholm 1993).

Without giving evidence, Oshima et al. (1969) regarded the developing stage of *Anisakis* sp. occurring in euphausiaceans as the second stage. The ecdysis to the third-stage larva was assumed to occur after the larva was ingested by a fish host. Smith (1983*b*) assumed that ensheathed second-stage larvae of *A. simplex* are ingested by euphausiaceans where they develop into third-stage larvae. He regarded three small larvae (4.2–5.9 mm long) from naturally infected euphausiaceans to be second-stage larvae in the process of moulting to the third stage. The anterior ends of these assumed second-stage larvae were similar to those of third-stage larvae; the ventriculus was relatively slim and did not appear to meet the intestine in an oblique manner. Smith (1983*b*) found a mucron, like that of third-stage larva, on an inner cuticle, but he did not verify this finding on photomicrographs. He concluded that larval *A. simplex* begins to moult from second- to third-stage larva when 4–6 mm long and does so in euphausiaceans. However, these small larvae are obviously young third-stage larvae, and the small morphological differences may be explained by the fact that the ventriculus is not yet fully developed.

The second ecdysis of *P. decipiens* has not previously been observed. McClelland and Ronald (1974*a*) cultured, in vitro, presumed second-stage larvae from hatched eggs that grew without moulting to a length of 33.3 mm. They were morphologically similar to larvae from cod. The fact that these presumed second-stage larvae grew to the infective third stage without further moult was explained by the fact that they were not exposed to physical or chemical stimuli that they might encounter on passage through one or more intermediate host(s). They observed gradual growth and definition of internal structures, and the same cuticular structures, boring tooth and papillae, were present in both the presumed second-stage larvae and infective third-stage larvae. Also, *C. osculatum* developed directly from the presumed second stage to the infective stage in vitro (McClelland and Ronald 1974*b*). Infective larvae from eggs of *H. aduncum* grew continuously without moulting to infective third-stage larvae in experimentally infected copepods (Køie 1993*a*). These studies by McClelland and Ronald (1974*a*, 1974*b*) and Køie (1993a) confirm the observation that the two first ecdyses take place in the egg.

Whereas the life cycle of *P. decipiens* is relatively well known (McClelland 1990), that of *A. simplex* is not known in detail. The importance of, e.g., a copepod transport host, is unknown (Smith 1983*b*; Brattey and Clark 1992; Køie 1993*b*), but the finding that the two first moults occur in the egg together with the knowledge that the two last moults occur in the definitive host should facilitate the elucidation of the life cycle. It is obvious that the infectivity of *A. simplex* and *P. decipiens* to the different suitable hosts in their life cycles depends on the size of the third-stage larvae only, similar to what has been found for *H. aduncum* (see Køie 1993*a*).

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Experimental infection of fish with larval sealworm, *Pseudoterranova decipiens* (Nematoda, Anisakinae), transmitted by amphipods

G. McClelland

Abstract: Larval sealworm (*Pseudoterranova decipiens*, (Krabbe 1878)) were transmitted sequentially through copepods and amphipods to 12 phylogenetically diverse fish species. Although *P. decipiens* in amphipods were 0.82–7.08 mm long, only those ≥1.41 mm were infective to fish. Sealworm transmitted at 15°C migrated to the musculature within 6 h in mummichog (*Fundulus heteroclitus*) and 12 h in rainbow smelt (*Osmerus mordax*) but were confined to the bolus in an Atlantic cod (*Gadus morhua*) stomach at 24 h. All sealworm in smelt, but only 12% of those in cod, occupied the musculature after 7 days. Death of 15 mummichog and a grubby (*Myoxocephalus aenaeus*) 10–20 days after exposure apparently resulted from damage to the brain, heart, aorta, liver, or spleen by feeding and migrating nematodes. Whereas 100% of the sealworm in grubby and 86–88% of those in mummichog and winter flounder (*Pleuronectes americanus*) were dead and encapsulated in host connective tissue after 56 days, all *P. decipiens* in smelt were viable and unencapsulated. With length increasing linearly at 0.421 mm day^{-1}, sealworm larvae reached 27.29 (23.60–30.70) mm after 56 days in smelt at 15°C. However, *P. decipiens*, which escaped the host tissue response in mummichog and flounder, were only 10.14 (6.00–21.80) and 12.42 (9.53–14.40) mm in length, respectively.

Résumé : Des larves de ver de phoque, *Pseudoterranova decipiens* (Krabbe, 1978), ont été transmises de façon séquentielle, par l'intermédiaire de copépodes et d'amphipodes, à des poissons appartenant à douze espèces phylogénétiquement diverses. Chez les amphipodes, les *P. decipiens* mesuraient de 0,82 à 7,08 mm de longueur, mais seuls ceux de ≥1,41 mm infestaient les poissons. Les vers de phoque transmis à 15°C se retrouvaient dans la musculature au bout de 6 h chez le choquemort (*Fundulus heteroclitus*) et de 12 h chez l'éperlan arc-en-ciel (*Osmerus mordax*) mais étaient confinés au bol alimentaire dans l'estomac d'une morue franche (*Gadus morhua*) au bout de 24 h. Tous les vers de phoque chez l'éperlan, mais seulement 12% chez la morue, occupaient la musculature au bout de 7 jours. La mort de 15 choquemorts et d'un crapaud de mer nain (*Myoxocephalus aenaeus*) 10 à 20 jours après l'exposition semblait imputable aux dommages causés au cerveau, ou coeur, à l'aorte, au foie ou à la rate par l'alimentation et la migration des nématodes. Tandis que 100% des vers de phoque chez le crapaud de mer nain, et 86–88% chez le choquemort et la plie rouge (*Pleuronectes americanus*), étaient morts et encapsulés dans le tissu conjonctif des hôtes au bout de 56 jours, tous les *P. decipiens* chez l'éperlan étaient viables et non encapsulés. Leur longueur augmentant de façon linéaire au rythme de 0,421 mm·jour^{-1}, les larves de ver de phoque atteignaient 27,29 (23,60–30,70) mm au bout de 56 jours chez l'éperlan à 15°C. Toutefois, les *P. decipiens* qui échappaient à la réaction du tissue des hôtes chez le choquemort et la plie ne mesuraient respectivement que 10,14 (6,00–21,80) et 12,42 (9,53–11,40) mm de longueur.

[Traduit par la Rédaction]

Introduction

Knowledge of the role that marine benthic invertebrates play in the life cycle of sealworm (*Pseudoterranova decipiens*, Krabbe 1878) has grown considerably in recent years. Laboratory studies (McClelland 1982; 1990) reveal that sealworm may be transmitted to benthic copepods (Harpacticoida and Cyclopoida) and larval gammaridean amphipods by direct exposure to newly hatched larvae, and to various benthic macroinvertebrates (polychaetes, nudibranchs,

Received March 15, 1994. Accepted December 15, 1994.
J12591

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mysids, cumaceans, mature amphipods, isopods, and decapods) through copepod carrier hosts. Larval *P. decipiens* 1.1–9.0 mm in length have also been described or tentatively identified from numerous naturally infected marine macroinvertebrates including a polychaete (Val'ter and Popova 1974), a gammarid (Val'ter 1987), and a caprellid (Val'ter 1978) from the White Sea, a decapod from the Barents Sea (Uspenskaya 1963), and four gammaridean amphipod (McClelland 1990; Marcogliese 1993*a*, 1993*b*) and three mysid species (Scott and Black 1960; Martell 1992; Marcogliese 1992*a*, 1993*a*; Marcogliese and Burt 1993) from eastern Canadian waters. In light of results from recent surveys (Paggi et al. 1991; Bristow and Berland 1992) in which sealworm collected throughout the North Atlantic and adjacent waters have been characterized biochemically by electrophoretic procedures, however, the nematodes from Arcto-Norwegian and Russian macrofauna and those from crustaceans collected in temperate waters off eastern Canada may belong to different, anatomically similar but reproductively isolated, sibling species.

Despite their small size, sealworm infecting natural and experimental macroinvertebrate hosts have structural characteristics of infective third-stage larvae and those >5 mm in length may be capable of moulting to fourth-stage larvae in the stomach of a definitive host (McClelland 1990, McClelland and Ronald 1974). Although *P. decipiens* larvae found in surveys of marine fish are usually much larger, e.g., 15–58 mm in length according to Templeman et al. (1957), larvae similar in size (4–9 mm in length) to those found in macroinvertebrate hosts have been detected in natural fish hosts such as Atlantic cod (*Gadus morhua*) from eastern Canadian (Scott 1956; McClelland et al. 1983*a*) and Icelandic waters (Pálsson 1979) and European smelt (*Osmerus eperlanus*) from the Elbe River, Germany (Möller and Klatt 1990). Hence, it is reasonable to assume that small sealworm larvae carried by benthic macrofauna are infective to fish. The present study documents serial transmissions of *P. decipiens* larvae through copepods and amphipods to fish hosts of diverse size and phylogeny. The subsequent course of infection in fish hosts is also described.

Materials and methods

Gravid female *Pseudoterranova decipiens* were collected from experimentally infected grey seals (*Halichoerus grypus*) housed in the Aquatron Laboratory, Dalhousie University, Halifax, N.S. (McClelland 1980) and from free-living grey seals from the Northumberland Strait, Cape Breton Island, and the eastern Nova Scotia mainland. Mature nematodes from free-living seals and larval nematodes transmitted to captive seals were collected in areas where only *P. decipiens* sibling B is known to occur (Paggi et al. 1991; Brattey and Stenson 1993). Procedures for incubation of ova and for collection, maintenance, and exposure of crustaceans were similar to those described in earlier studies (McClelland 1982; 1990). Ova dissected from the vagina and uterus of the nematodes were transferred to finger bowls containing 250 mL of fresh prefiltered seawater and incubated at 5 and 15°C. Development of ova and condition of ensheathed larvae emerging from ova were monitored weekly at 5°C and daily at 15°C. Approximately 80% of the seawater in finger bowls was replaced during each monitoring period. Marine benthic and epibenthic crustaceans including harpacticoid (*Halectinosoma* sp., *Tisbe* spp., and *Ameira longipes*) and cyclopoid (*Paracyclopina* sp.) copepods and gammaridean amphipods (*Gammarus lawrencianus* and *Unciola irrorata*) were collected from the Northwest Arm, Halifax. Two hundred copepods and 20 000 ± 2000 recently hatched *P. decipiens* larvae were placed in 4-L jars of fresh prefiltered seawater at 15°C with a thin sediment (1–2 mm deep) of fine organic detritus from the Northwest Arm. The sediment and the microflora and microfauna therein provided a source of food for the copepods as well as a substrate to which the nematodes could adhere. Twenty amphipods were placed in 4-L jars of seawater at 15°C with 200 copepods that had previously been exposed to newly hatched *P. decipiens* larvae for 24 h. Amphipods were exposed to infected copepods for 24 h to 7 days and maintained at 15°C on a diet of finely ground Tetramin™ Staple Food for periods of up to 56 days from the beginning of exposure to the parasite (PE). Approximately 80% of the seawater was replaced daily.

Atlantic salmon parr (*Salmo salar*) and juvenile brook trout (*Salvelinus fontinalis*) used as experimental hosts for *P. decipiens* were hatchery stock obtained from the aquarium at the Department of Fisheries and Oceans, Halifax Fisheries Research Laboratory. Alewife (*Alosa pseudoharengus*), mummichog (*Fundulus heteroclitus*), threespine stickleback (*Gasterosteus aculeatus*), cunner (*Tautogolabrus adspersus*), Atlantic silverside (*Menidia menidia*), grubby (*Myoxocephalus aenaeus*), and juvenile winter flounder (*Pleuronectes americanus*) were collected by beach seine at various locations in eastern Halifax County including Petpeswick Inlet, Lower Porter's Lake, and the Lawrencetown salt marsh. Rainbow smelt (*Osmerus mordax*) were taken by dip net from a spawning brook at the head of Petpeswick Inlet, while Atlantic cod and pollock (*Pollachius virens*) were obtained from mackerel traps in St. Margarets Bay, southwestern Halifax County. Fish were transferred to 1.5 × 1.5 × 1.2 m and 0.8 × 0.5 × 0.3 m fiberglass aquaria. Aquaria containing marine and estuarine species were provided with a continuous flow of fresh prefiltered seawater at ambient temperature, which varied from 2°C in midwinter to 17°C in late summer and fall. Flow rates were set at 0.3–1.0 $L \cdot min^{-1}$ according to capacity and biomass contained. Aquaria holding juvenile salmon and trout were supplied with a continuous flow (0.3 $L \cdot min^{-1}$) of dechlorinated tapwater and maintained at 10°C in a thermostatically controlled room. With the exception of smaller species such as mummichog, stickleback, and silverside which were fed Tetramin™ Staple Food, fish consumed frozen alewife, Atlantic herring (*Clupea harengus*), white perch (*Morone americana*), and mackerel (*Scomber scombrus*), which were first cut into pieces of appropriate size and thawed.

Recently hatched *P. decipiens* larvae (n = 50–150) or copepods (n = 10–20), which had previously been exposed to the nematodes, were fed to cod, grubby, and flounder by oral intubation in 1–2 mL of 9 ppt seawater with 3.2 mm I.D. Tygon™ tubing affixed to a 10-mL disposable syringe.

In the remaining transmissions, fish consumed infected crustaceans voluntarily. Transmissions to small fish such as larval smelt, mummichog, and stickleback were conducted in 4-L plastic buckets in which individual fish were isolated either with 10–20 copepods or with 2–5 amphipod precursor hosts. Larger fish including mature smelt, silverside, and grubby and juvenile salmon, trout, cod, etc. were isolated individually in 0.8 × 0.5 × 0.3 m tanks with two to five experimentally infected amphipods or two to five mummichog, that had previously consumed infected amphipods. As it was possible to count *P. decipiens* larvae in fresh, lightly infected (intensity = 1–3) *G. lawrencianus* using a stereo microscope, the numbers of nematodes in amphipods fed to individual trout, smelt, mummichog, and stickleback were known in some instances. Fish were exposed and subsequently held for periods of up to 8 weeks in temperature-controlled rooms at 5 and 10°C, in holding tanks in the main wet lab (above) at ambient seawater temperature (10–17°C) and in 0.8 × 0.5 × 0.3 m aquaria at 15 ± 1.0°C. Fish exposed individually but simultaneously to identical numbers of infected crustaceans were usually maintained in common holding tanks after exposure.

Fish that had been exposed to crustaceans (or mummichog) infected with *P. decipiens* were sampled at 3, 6, 12, and 24 h PE and weekly from 7 to 56 days PE. These specimens, as well as fish that died subsequent to exposure and those serving as unexposed controls, were examined for parasitic nematodes by dissection and inspection of tissue squashes using a stereo microscope. All nematodes detected were identified, counted, preserved in hot glycerin–alcohol, and cleared by evaporation in glycerin. Preserved *P. decipiens* larvae from experimentally infected fish were used in morphometric comparisons with larval sealworm grown in vitro (in Eagle's MEM, supplemented with foetal calf serum) during an earlier study (McClelland and Ronald 1974). Body lengths of nematodes were measured using a stereomicroscope equipped with a drawing tube and stage micrometer, while dimensions or positions of characteristic structures were determined using a compound microscope with the aid of ocular and stage micrometers. Parasitized tissue samples from experimental fish hosts were preserved in buffered 10% formalin and routinely dehydrated, cleared, embedded, sectioned at 5 μm, and stained with haematoxylin and eosin.

SYSTAT ANOVA, ANCOVA, and MANCOVA procedures (Wilkinson 1989) were used in comparisons of nematode lengths and in analysis of nematode growth and morphometry. Significance was set at the 1% probability level. Quantitative terms such as prevalence (*P*), abundance (*A*) and intensity (*I*) are defined according to Margolis et al. (1982).

Results

Experimental transmissions of *P. decipiens* to fish

Newly hatched larvae of *P. decipiens* (200–215 μm in length, heat-relaxed) intubated orally to two cod and five grubbies failed to exsheathe and penetrate the gut wall. Similarly, larger (220–375 μm) exsheathed larvae from the haemocoels of copepods intubated to or voluntarily consumed by cod (n = 2), mummichog (n = 10), threespine stickleback (n = 10), and grubby (n = 5) were subsequently found only in the gastrointestinal lumen where they persisted for as long as 48 h at 15°C and 7 days at 5–10°C before being passed with the faeces.

Experimental transmissions of *P. decipiens* to mummichog using amphipods with infections of <28 days duration at 15°C revealed that sealworm larvae <2 mm in length seldom penetrated the gut wall of fish. The smallest larvae infecting mummichog exposed to amphipods with 56-day-old sealworm infections were 1.41 and 1.82 mm in length, respectively, at 24 h PE. Both of these nematodes were found in the gut wall and were the only sealworm larvae (2% of 94 recovered from mummichog tissues) <2 mm in length. In contrast, 30 (9%) of 335 *P. decipiens* from a control sample (n = 50) of amphipods were <2 mm in length.

Larval *P. decipiens* were found in the body cavities and skeletal musculature of 11 species of fish that had previously consumed two to five amphipod precursor hosts with 56-day-old infections (Table 1). The parasite was also recovered from the body cavities and flesh of smelt, cod, and pollock following consumption of two to five mummichog, each of which had ingested two infected amphipods 7–14 days earlier. As revealed by transmissions in which larval sealworm dosages were known through prior microscopical examinations of lightly infected (I = 1-3) *Gammarus*, most nematodes from 56 days infections in amphipods successfully invaded the tissues of experimental fish hosts. The proportion of nematodes transmitted ranged from 60% in brook trout to 100% in mummichog (Table 1). Transmissions involving randomly selected amphipods seemed equally efficient. After consuming randomly selected *Gammarus* and *Unciola* with a mean sealworm count of 6.70 (I = 1–63) (n = 50), cod (n = 2), mummichog (n = 50), grubby (n = 7), and flounder (n = 4) were infected with 5.60, 5.68, 7.00, and 8.75 *P. decipiens* larvae, respectively, for each amphipod ingested. When worm count distributions in amphipods and mummichog are compared (Fig. 1), however, it appears that not all nematodes from heavily infected amphipods were successfully transmitted to fish. Both distributions are positively skewed with peak frequency in the 1-5 nematode range but, whereas total counts in 10 (20%) of 50 randomly selected amphipods pairs exceed 30 nematodes, the tail of the distribution in mummichog is compressed with a maximum intensity of 30 and a secondary peak in the 11–15 nematode range.

Because of a crowding effect in which length of *P. decipiens* in amphipods declines with increasing intensity of infection (McClelland 1990), nematodes transmitted to fish by heavily infected amphipods were smaller than those transmitted by lightly infected amphipods. Sealworm larvae from *Gammarus* and *Unciola* with light (I = 1-3) (n = 32), moderately heavy (I = 4–10) (n = 8), and heavy infections ($I \geq 11$) (n = 10) were 5.59 (3.89–7.08) (n = 60), 4.31 (3.20–6.03) (n = 57), and 2.90 (0.82–5.18) (n = 218) mm in length, respectively. Thirty (14%) of 218 nematodes from heavily infected amphipods were >2 mm in length and, as indicated above, *P. decipiens* of this size were probably not infective to fish.

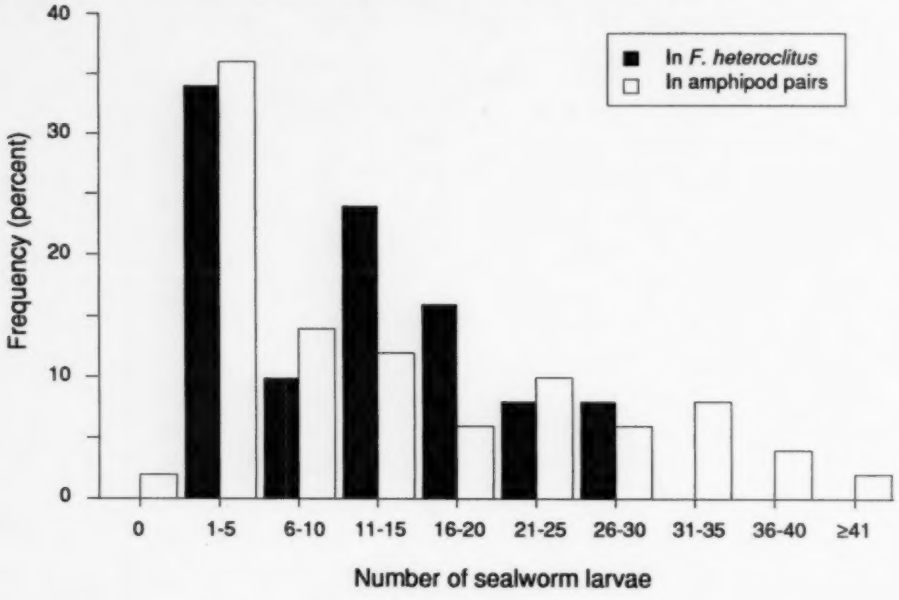
Sealworm larvae were not detected in control samples (n = 50) of alewife, salmon parr, brook trout, mummichog,

Table 1. Serial transmissions of larval sealworm (*Pseudoterranova decipiens*) to individual fish hosts via the amphipods *Gammarus lawrencianus* (G) and *Unciola irrorata* (U) and mummichog, *Fundulus heteroclitus* (F).

Experimental fish host				Precursor host		*P. decipiens* transmitted to fish			
Order / family / species	Common name	Body length range (cm)	*n*	Species	No. ingested by each fish	Total no. ingested	Total no. in fish tissues	% transmitted	Mean intensity (range)[a]
Clupeiformes									
Clupiedae									
Alosa pseudoharengus	Alewife	5–6	5	G, U	1		11		2.20 (1–4)
Salmoniformes									
Salmonidae									
Salmo salar	Atlantic salmon	8–12	3	G, U	2		21		7.00 (3–12)
Salvelinus fontinalis	Brook trout	10–15	5	G	2[b]	20	12	60	2.40 (1–4)
Osmeridae									
Osmerus mordax	Rainbow smelt	13–18	15	G, U	2		79		5.27 (3–11)
			5	G	5[b]	49	42	86	8.40 (5–9)
			5	F	2		73		14.60 (8–25)
Gadiformes									
Gadidae									
Gadus morhua	Atlantic cod	30–50	4	G, U	5		112		28.00 (19–44)
			2	F	5		89		(32,57)
Pollachius virens	Pollock	30–50	2	F	5		49		(28,21)
Atheriniformes									
Cyprinodontidae									
Fundulus heteroclitus	Mummichog	5–8	50	G, U	2		568		11.36 (2–30)
			10	G	1–3[b]	38	38	100	3.80 (2–6)
Atherinidae									
Menidia menidia	Atlantic silverside	10–12	5	G, U	2		30		6.00 (3–9)
Gasterosteiformes									
Gasterosteidae									
Gasterosteus aculeatus	Threespine Stickleback	4–6	10	G	1[b]	18	17	94	1.70 (1-3)
Perciformes									
Labridae									
Tautogolabrus adspersus	Cunner	10–12	5	G, U	2		16		3.20 (1–8)
Cottidae									
Myoxocephalus aenaeus	Grubby	9–13	7	G, U	5		245		35.00 (10–75)
Pleuronectiformes									
Pleuronectidae									
Pleuronectes americanus	Winter flounder	10–20	4	G, U	5		175		43.75 (24–58)

[a]Prevalence of *P. decipiens* in experimental fish hosts was 100%.
[b]Nematodes in haemocoels of lightly infected (I = 1–3) amphipods (*Gammarus lawrencianus*) counted prior to transmission.

Fig. 1. Frequency distributions of larval *Pseudoterranova decipiens* counts in 50 *Fundulus heteroclitus* 7–56 days after each had consumed two infected amphipods, and in 50 randomly selected pairs of infected amphipods (*Gammarus lawrencianus* and *Unciola irrorata*).



stickleback, and silverside nor were they found in samples (n = 10) of unexposed pollock, cunner, grubby, and winter flounder. However, 5 of 50 smelt examined at time of capture and 4 of 50 unexposed smelt inspected after 40–50 weeks in captivity were infected with single sealworm larvae ranging from 36.0 to 38.8 mm in length. Larval sealworm 27.1–40.2 mm in length were also found in 4 of 10 cod (I = 1–7) examined at time of capture and in 3 of 10 control cod (I = 1–3) after 15–50 weeks in captivity. Two sealworm larvae, 36.2 and 36.8 mm in length, from experimentally infected smelt at 14 and 42 days PE, respectively, were deemed to be of natural origin while nematodes 7.21–13.00 mm (n = 15), 15.80–24.30 mm (n = 11) and 23.60–30.70 mm (n = 16) in length after respective intervals of 14, 42, and 56 days PE in smelt were believed have to come from experimental transmissions. Similarly, of 206 *P. decipiens* larvae recovered from six cod at 7 days PE, 201 specimens <8 mm in length were probably from experimental transmissions, while the remaining five nematodes exceeded 30 mm in length and were clearly natural infections.

Distribution of *P. decipiens* larvae in fish host tissues

Time required for *P. decipiens* to penetrate the gut wall and migrate to the skeletal musculature of experimental fish hosts at a given temperature varied with size and species of host. At 15°C, sealworm larvae penetrated to the body cavities of mummichog and smelt by 3 h PE and were found in the adjacent hypaxial musculature of these species by 6 and 12 h PE (Table 2). Heavily infected amphipods with sealworm larvae protruding from their exoskeletons were regurgitated by smelt at 1.5–2.0 h PE, however, and this seemed to be indicative of the earliest penetration of the stomach wall. Sealworm reached the hypaxial and epaxial musculature by 24 h PE in all fish species exposed and maintained at 15°C with the exception of the largest experimental hosts, cod and pollock. At 15°C larval sealworm transmitted to cod were confined to the food bolus at 24 h PE and only 24 (12%) of 201 and 2 (4%) of 49 nematodes occurred in the flesh of cod and pollock, respectively, at 7 days PE. At 5–10°C, invasion of the tissues of even the smallest experimental hosts was delayed for 3–7 days.

The first *P. decipiens* larvae to migrate to the body cavity and flesh of fish were usually the largest (Fig. 2). Sealworm larvae found enterically and in the wall of the upper intestine (*Fundulus* spp. lack a stomach) of 10 mummichog at 24 h PE were 2.41 (1.38–4.10) (n = 16) mm in length and, according to ANOVA, significantly smaller than those in the body cavity and skeletal musculature. Nematodes from the body cavity and flesh were 3.53 (2.42–5.50) (n = 46) and 4.23 (2.73–6.20) (n = 38) mm in length, respectively. Twenty (63%) of 32 sealworm >4.00 mm in length, 16 (44%) of 36 nematodes 3.01–4.00 mm in length, but only 2 (6%) of 32 worms <3.00 mm in length occupied the flesh. Whereas larger (≥3.01 mm in length) sealworm larvae usually migrated to the body cavity via the stomach or upper intestinal wall within 3–12 h PE at 15°C, smaller (≤3.00 mm in length) nematodes were found penetrating the walls of the lower intestine at 24 h to 7 days PE. In cod examined at 7 days PE, larval sealworm had penetrated the alimentary canal throughout its length from oesophagus to rectum but occurred in greatest density (29 of 78 nematodes embedded in the gut wall) in the rectal wall. Larval *P. decipiens* migrating through the body

Table 2. Distribution of *Pseudoterranova decipiens* larvae in the tissues of experimental fish hosts exposed and maintained at 15 ± 1°C, 3 h to 8 weeks post exposure (PE).

Host		Time (h) (PE)	Total no. of sealworm larvae recovered (moribund nematodes in parentheses)	Distribution (%) of nematodes in fish hosts (moribund nematodes in parentheses)			
Species	*n*			Enteric	Gut wall	Body cavity and viscera	Skeletal muscle
Salmo salar	2	24	17	18	18	47	18
	3	170	21	—	—	21	81
Osmerus mordax	2	3	29	48	45	7	—
	2	12	22	23	23	50	5
	2	24	15	—	20	47	33
	2	170	11	—	—	—	100
	4	1350	16	—	—	—	100
Gadus morhua	1	24	37	100	—	—	—
	6	170	201	—	39	49	12
Pollachius virens	2	170	49	—	36	60	4
Fundulus heteroclitus	5	3	68	47	34	19	—
	5	6	44	25	32	41	2
	5	12	60	18	20	53	8
	10	24	100	6	12	44	38
	10	170	111	—	—	58	42
	15	670	183 (57)	—	—	70 (91)	30 (9)
	20	1350	243 (210)	—	—	69 (75)	31 (25)
Myoxocephalus aenaeus	2	24	66	5	11	61	23
	2	170	23	—	—	53	47
	2	1350	87 (87)	—	—	27 (27)	73 (73)
Pleuronectes americanus	1	24	58	32	21	36	11
	2	170	75	—	29	35	36
	1	1350	42 (37)	—	—	81 (89)	19 (11)

cavities of fish not only occupied visceral epithelia and mesenteries but were often found deeply embedded in larger organs such as the liver and spleen.

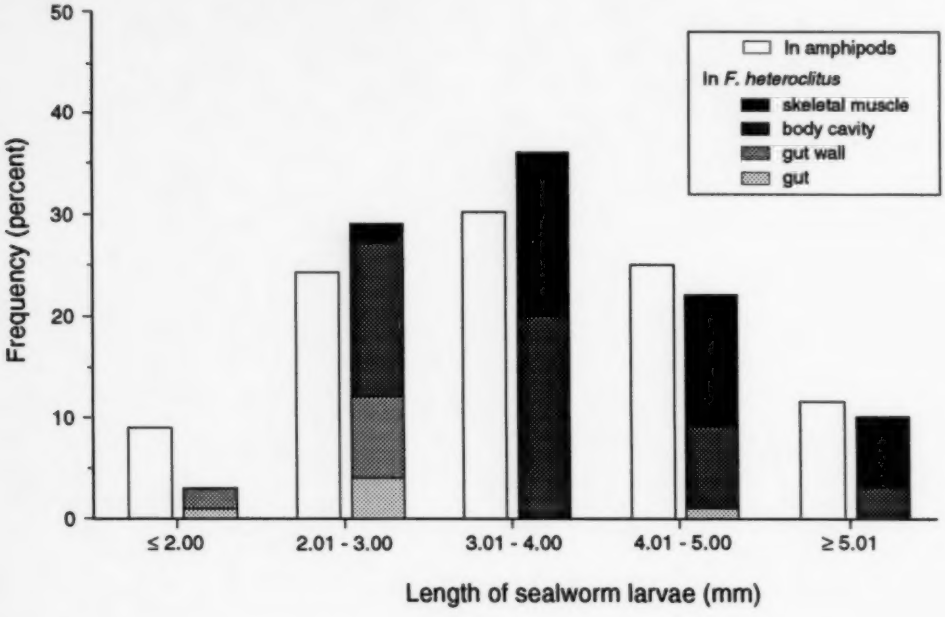
Distribution of the nematodes between the body cavity and somatic musculature varied with host species (Table 2). All sealworm larvae ($n = 75$) in 14 smelt at 7–56 days PE were recovered from the hypaxial and epaxial musculature, and the majority (17 of 21) of those found in salmon at 7 days PE, and in grubby (63 of 87) at 56 days PE also occupied the flesh. In contrast, 553 (67%) of 820 *P. decipiens* larvae in 70 mummichog at 7–56 days PE, and 34 (81%) of 42 sealworm in a winter flounder at 56 days PE occurred in the body cavity. A small proportion (<5 %) of the nematodes infecting mummichog had aberrantly penetrated the pericardium, dorsal aorta, or brain or lay subcutaneously on the jaw, in the orbit or elsewhere on the head.

Host–parasite interactions

Mortalities among fish exposed to larval *P. decipiens* were confined to 15 mummichog (21% of 70 with infections >7 days duration) and a single grubby which died overnight within 10–20 days PE. Subsequent examinations revealed that the grubby and seven mummichog appeared to have suffered mechanical damage to the liver or spleen attributable to the migration and feeding of numerous sealworm larvae in the body cavity; 34 sealworm were recovered from the body cavity of the grubby, 11–23 from the body cavities of the mummichog. Mortality of eight mummichog, however, seemed attributable to penetration of the brain (five cases), pericardium (two cases), or dorsal aorta (one case).

There was marked deposition of fat in the body cavities of mummichog which survived heavy sealworm infection ($I > 10$) for 14–56 days. Fatty connective tissue encapsulated not only those nematodes lying on visceral epithelia, mesenteries and peritonea, but also those embedded in visceral organs and adjacent hypaxial musculature. After 28 days in mummichog ($n = 15$), 57 (31%) of 183 sealworm larvae were moribund (Table 2), including 36 (63%) of 57 encapsulated on or in the liver, 17 (26%) of 65 on the

Fig. 2. Length frequency distribution of 335 *Pseudoterranova decipiens* larvae after 56 days in amphipods (*Gammarus lawrencianus* and *Unciola irrorata*) at 15°C and distribution, by length and location in host, of 100 sealworm larvae from 10 *Fundulus heteroclitus* 24 h after exposure to infected amphipods.



mesenteries, and 4 (18%) of 22 in the abdominal wall (hypaxial musculature). Twenty-seven nematodes found in subcutaneous tissues of the head, in the dorsal (epaxial) musculature of the trunk, or in the caudal (epaxial and hypaxial) musculature were viable and unencapsulated. By 56 days PE, 210 (86%) of 243 *P. decipiens* larvae in 20 mummichog were dead and encapsulated but while only 11 (<7%) of 168 nematodes in the body cavity and 3 (<6%) of 55 in the abdominal musculature survived, each of the 19 sealworm larvae occupying cranial and caudal musculature was unencapsulated and robust.

Unlike *P. decipiens* larvae in mummichog, those found in the body cavities and flesh of two grubbies and a winter flounder at 56 days PE were encapsulated in fibrous connective tissue. All nematodes (n = 87) from the grubbies were encapsulated and moribund as were 37 (88%) of 42 worms from the winter flounder. Of five viable, unencapsulated sealworm larvae in the flounder, four occupied the fillets. There was no evidence of a host response to *P. decipiens* infection in smelt, however, and all nematodes (75) recovered from this host during a 56-day course of infection were active, robust, and unencapsulated.

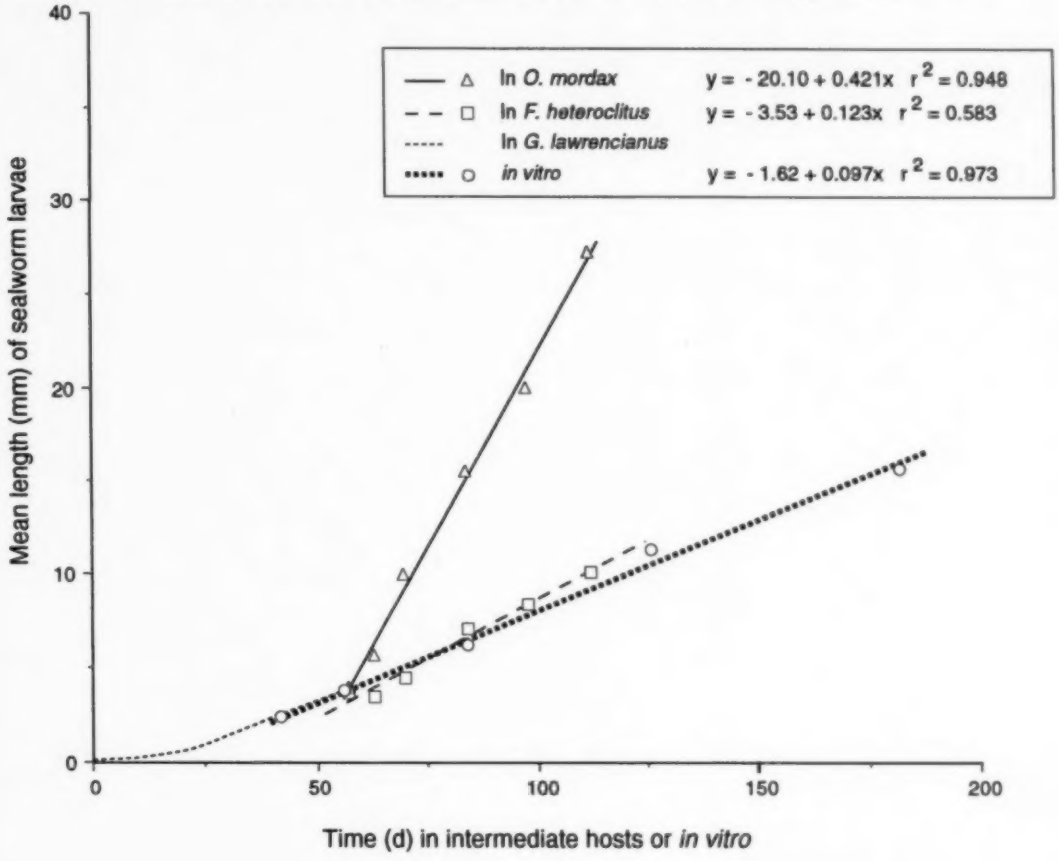
Viable, unencapsulated larvae of *P. decipiens* observed in fresh, tissue-squash preparations from fish maintained at 15°C were fully extended and actively ingesting host tissue fluids or blood, while encapsulated worms were fully extended to partially coiled and inactive. Both encapsulated and unencapsulated sealworm larvae in the tissues of fish maintained at <10°C were usually coiled and often formed compact spirals.

Growth of *P. decipiens* larvae in fish

Larval *P. decipiens* found in a control sample (n = 50) of amphipods after 56 days PE at 15°C were 3.62 (0.82–7.08) (n = 335) mm in length, while those recovered from the gut wall, body cavities, and skeletal musculature of smelt (n = 2) and mummichog (n = 20) 24 h after consumption of infected amphipods were 3.89 (2.77–6.05) (n = 15) and 3.68 (1.41–6.18) (n = 94) mm in length, respectively. With body length increasing at a linear rate (Fig. 3) nematodes in smelt (n = 4), mummichog (n = 20), and winter flounder (n = 1) grew to respective lengths of 27.29 (23.60–30.70) (n = 16), 10.14 (6.00–21.80) (n = 33 viable nematodes), and 12.42 (9.53–14.40) (n = 5 viable nematodes) after 56 days PE at 15 ± 1° C; however, encapsulated, nonviable sealworm from the skeletal muscle of grubby (n = 2) at 56 days were only 4.41 (3.15–5.53) (n = 40) mm in length. After an initial exponential growth phase, *P. decipiens* larvae reared in vitro at 15°C (McClelland and Ronald 1974) also grew linearly in body length from 2.38 (2.00–3.06) (n = 20) mm after 42 days to 11.30 (9.92–13.00) (n = 20) mm after 126 days and to 21.90 (18.90–24.20) (n = 20) mm after 238 days of cultivation (Fig. 3).

ANCOVA indicated that growth of *P. decipiens* larvae in fish hosts maintained for 56 days PE at 15 ± 1° C was significantly more rapid in smelt (0.421 mm·day^{-1}) (n = 90) than in mummichog (0.123 mm·day^{-1}) (n = 273). Further, the growth rate of sealworm larvae in smelt significantly exceeded that observed (0.097 mm·day^{-1}) (n = 60) for larvae developing in vitro at 15° C over a corresponding period of time (from day 56 to day 126). Growth rates of

Fig. 3. Relationships of the length of larval *Pseudoterranova decipiens* in *Osmerus mordax* (n = 90), *Fundulus heteroclitus* (n = 273), and in vitro (n = 60) (McClelland and Ronald 1974) to time (days) at 15°C.



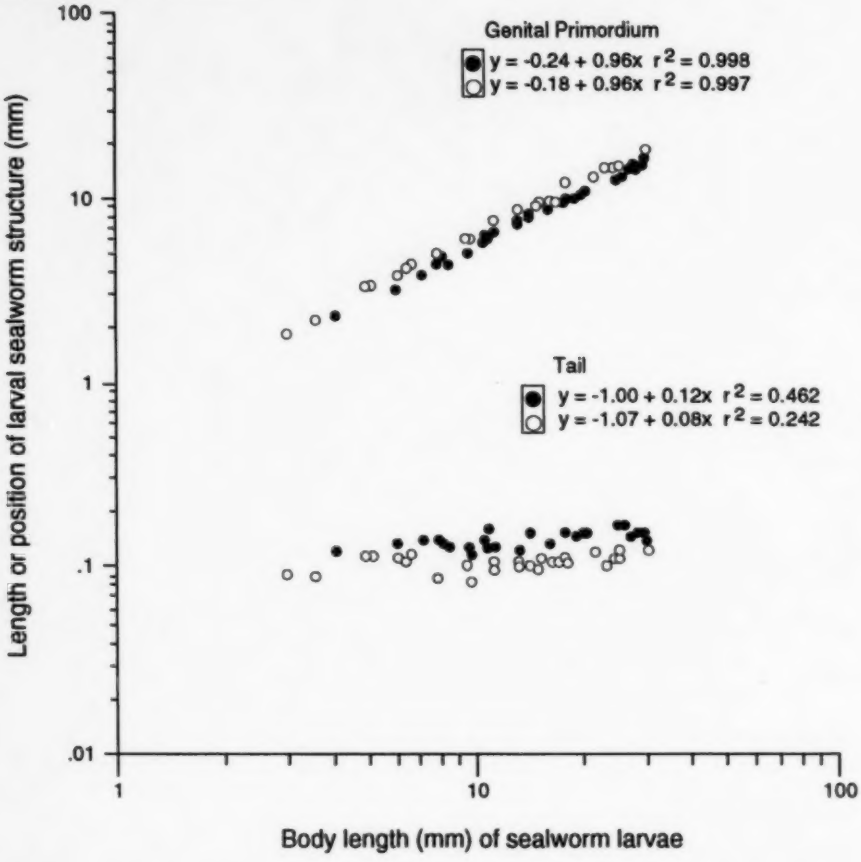
sealworm in mummichog and in vitro, however, did not differ significantly. ANCOVA also revealed that *P. decipiens* recovered from mummichog after 57–112 days did not differ in length from those growing in vitro for 56–126 days.

Structure of *P. decipiens* larvae from fish

Larval *P. decipiens* from experimentally infected fish had the characteristics of third-stage larvae (L_3s). Primordia of the three bilobed lips of the fourth stage larva (L_4) were distinctly visible beneath the cephalic cuticle in en face preparations. The intestinal caecum, arising at the ventriculo-intestinal junction and extending anteriorly and dorsal to the ventriculus, was well developed although highly variable in length. Also apparent was the ramified ligament anchoring the distal end of the caecum to the hypodermis. Genital primordia were multicellular and sexually dimorphic and found in the posterior half of the nematode near mid-body. The proximal end of the female primordium lay in the ventral hypodermis, while the bilobed distal end extended posteriorly in the ventral pseudocoel. The male primordium, an elongate cylindrical structure with a recurved anterior end, lay in the sublateral hypodermis, parallel and ventral to the left lateral excretory canal. ANCOVAs indicated that male (n = 28) and female (n = 28) larvae differed significantly in respect to the position of the genital primordium and tail length, the genital primordium being found in a more anterior position and the tail being longer in female nematodes (Fig. 4).

Pseudoterranova decipiens larvae did not seem to moult in fish hosts. Neither moulting nematodes nor cast cuticles were found in the gut, gut wall, body cavity, or musculature of experimental hosts, or was there indirect evidence of moulting such as changes in the parasites' morphometry, which may be indicative of passage from one larval stage to the next. According to a MANCOVA, larval *P. decipiens* 2.78–7.21 mm in length (n = 20) after 7–14 days in experimental fish hosts were morphometrically similar to sealworm larvae 2.00–6.90 mm in length (n = 21) from amphipods with respect to the lengths of the preventriculus, ventriculus, caecum, and tail and the positions of the nerve ring and genital primordium (Fig. 5). Larvae 2.78–30.70 mm in length (n = 70) from experimentally infected fish also appeared to be morphometrically similar to sealworm larvae 2.00 to 33.90 mm in length (n = 70)

Fig. 4. Morphometric regressions for tail length and distance of genital primordium from anterior end in 28 female (●) and 28 male (○) larvae of *Pseudoterranova decipiens* from experimentally infected fish.



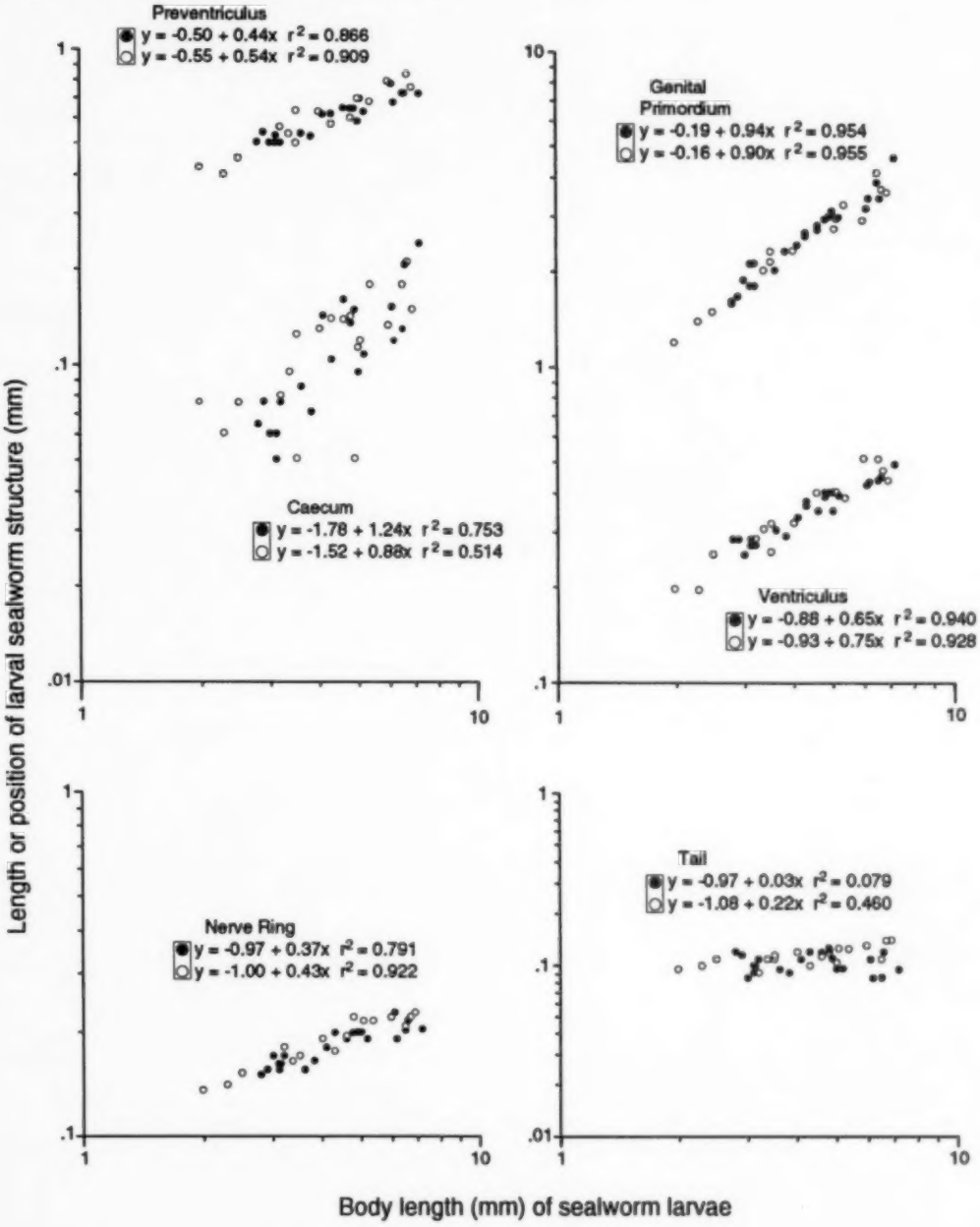
reared in vitro (McClelland and Ronald 1974) (Fig. 6), although ANCOVAs indicated the two groups of nematodes differed significantly with regards to slopes of regressions for position of the nerve ring and elevations of regressions for lengths of ventriculus and caecum.

Discussion

Results of experimental transmissions described herein indicate that marine macroinvertebrates, in this instance gammaridean amphipods, are essential intermediaries in the life cycle of the sealworm, *Pseudoterranova decipiens*. While neither freshly hatched ensheathed sealworm larvae nor exsheathed larvae occupying the haemocoels of benthic copepods were infective to fish hosts, larvae from chronic (28–56 days at 15°C) infections in amphipod haemocoels successfully invaded the body cavities and skeletal musculature of various marine, anadromous and freshwater fishes. According to Smith et al. (1990), newly hatched *P. decipiens* fail to establish themselves in rainbow trout (*Oncorhynchus mykiss*), even when implanted directly in the body cavity by intraperitoneal injection. Further, although sealworm larvae infecting benthic harpacticoids and cyclopoids had exsheathed and grown to 300–500 μm in length (McClelland 1982), the smallest larvae to penetrate the gut wall of experimental fish hosts in the present study were ≥1.41 mm and usually >2.00 mm in length. A *P. decipiens* larva recovered from an experimentally infected calanoid, *Tortanus discaudata*, was 2.5 mm in length (M.D.B. Burt, Department of Biology, University of New Brunswick, Fredericton, N.B., personal communication) but a planktonic calanoid would not normally consume the benthic newly hatched larvae of *P. decipiens* in nature and hence, seems an unlikely host. However, the nematode might be transmitted to fish by as yet unidentified benthic copepods that are of sufficient size and longevity to accommodate sealworm growth similar to that observed in *T. discaudata* (McClelland 1990).

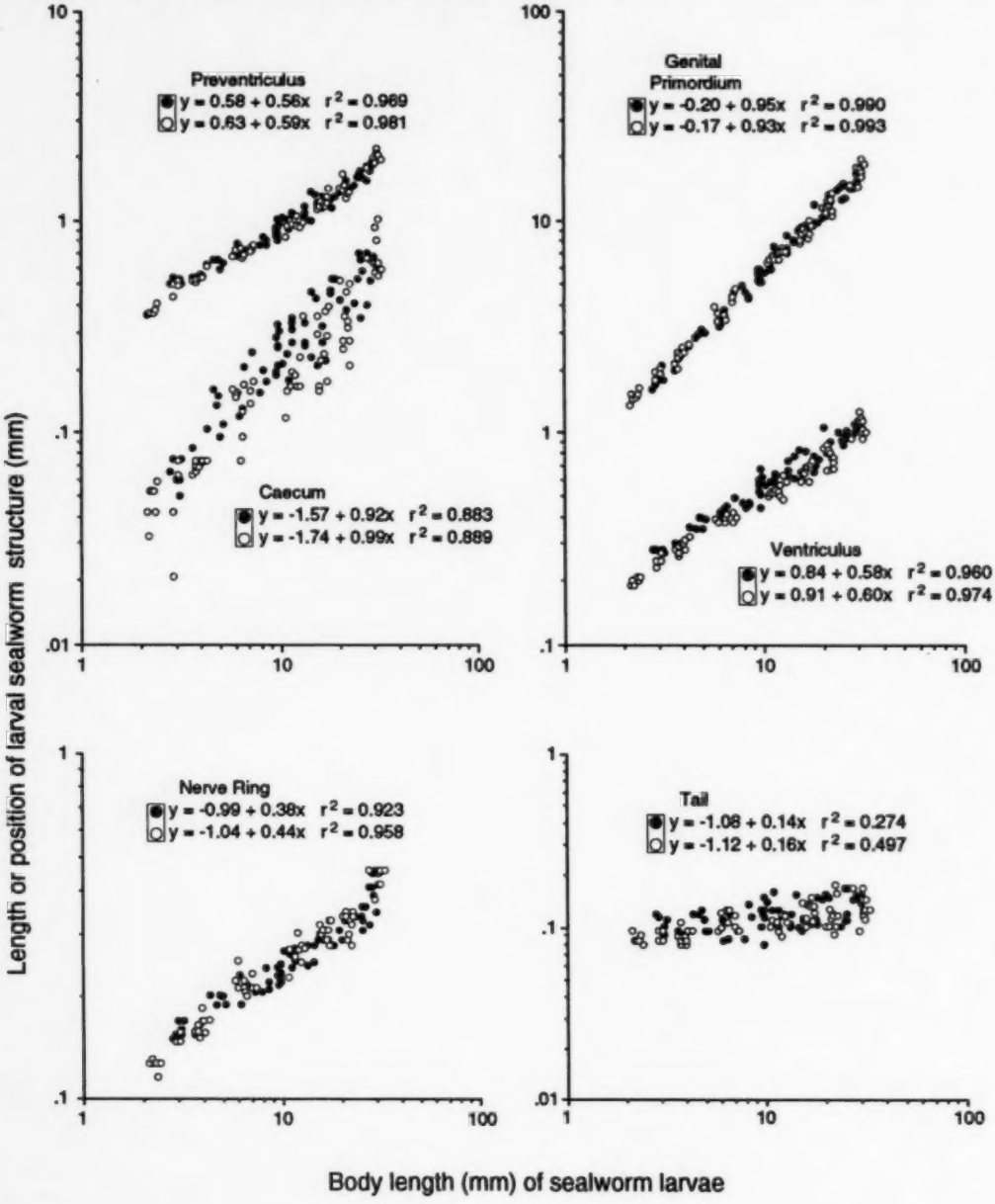
Although evidence of direct transmission of *P. decipiens* from copepods to fish is lacking, laboratory experiments (McClelland 1990) have shown that transmission of the parasite to juvenile amphipods, susceptible to infection by exposure to freshly hatched ensheathed larvae, is enhanced through the participation of copepod carrier hosts. Moreover,

Fig. 5. Morphometric regressions for lengths of preventriculus, ventriculus, caecum, and tail and for distances of nerve ring and genital primordium from the anterior end in *Pseudoterranova decipiens* larvae from 7 to 14 days infections in *Osmerus mordax* and *Fundulus heteroclitus* (●) (n = 20) and from 56 day infections in *Gammarus lawrencianus* (○) (n = 21).


Preventriculus
y = -0.50 + 0.44x r² = 0.866
y = -0.55 + 0.54x r² = 0.909
Caecum
y = -1.78 + 1.24x r² = 0.753
y = -1.52 + 0.88x r² = 0.514
Genital Primordium
y = -0.19 + 0.94x r² = 0.954
y = -0.16 + 0.90x r² = 0.955
Ventriculus
y = -0.88 + 0.65x r² = 0.940
y = -0.93 + 0.75x r² = 0.928
Nerve Ring
y = -0.97 + 0.37x r² = 0.791
y = -1.00 + 0.43x r² = 0.922
Tail
y = -0.97 + 0.03x r² = 0.079
y = -1.08 + 0.22x r² = 0.460
Length or position of larval sealworm structure (mm)
Body length (mm) of sealworm larvae

macroinvertebrates (polychaetes, nudibranchs, mysids, mature amphipods, isopods, cumaceans, and decapods) not susceptible to infection by ensheathed larvae are readily infected by larger exsheathed larvae from copepod haemocoels. Evidently, copepods are important prey for epi- or supra-benthic macrofauna such as mysids (Rudstam et al. 1989), which are natural hosts of larval sealworm in eastern Canadian waters (Scott and Black 1960; Martell 1992; Marcogliese 1992*a*, 1993*a*, 1993*b*; Marcogliese and Burt 1993).

Fig. 6. Morphometric regressions for lengths of preventriculus, ventriculus, caecum, and tail, and for distances of nerve ring and genital primordium from the anterior end in *Pseudoterranova decipiens* larvae after 7 to 56 days in fish (●) (n = 70) and 6–52 weeks in vitro (○) (n = 70).


Preventriculus
y = 0.58 + 0.56x r² = 0.969
y = 0.63 + 0.59x r² = 0.981
Caecum
y = -1.57 + 0.92x r² = 0.883
y = -1.74 + 0.99x r² = 0.889
Genital Primordium
y = -0.20 + 0.95x r² = 0.990
y = -0.17 + 0.93x r² = 0.993
Ventriculus
y = 0.84 + 0.58x r² = 0.960
y = 0.91 + 0.60x r² = 0.974
Nerve Ring
y = -0.99 + 0.38x r² = 0.923
y = -1.04 + 0.44x r² = 0.958
Tail
y = -1.08 + 0.14x r² = 0.274
y = -1.12 + 0.16x r² = 0.497
Length or position of larval sealworm structure (mm)
Body length (mm) of sealworm larvae

Gammaridean amphipods used as experimental hosts in this study are natural hosts of sealworm (McClelland 1990), but their significance in the transmission of the parasite is unknown. *Gammarus lawrencianus* is confined to shallow inshore waters, estuaries, and tidal pools, while *U. irrorata* is more widely distributed, being found from the lower intertidal zone to deeper (>60 m) offshore waters (Bousfield 1973). A recent study of the diets of offshore pleuronectids from waters near Sable Island, Canada (Martell and McClelland 1994) reveals that *U. irrorata* is principal prey of flatfishes such as American plaice (*Hippoglossoides platessoides*), which are heavily infected with sealworm (McClelland et al. 1987, 1990). Martell (1992) found the remains of several *U. irrorata* among other prey in the

stomach of a yellowtail flounder (*Pleuronectes ferrugineus*) which, perhaps not coincidentally, also contained five tiny (2.8–8.7 mm) *P. decipiens* larvae. Natural infections with sealworm larvae of similar size have also been reported in intertidal sandhoppers (*Americorchestia megalophthalma*) (Marcogliese 1993*a*) and sublittoral burrowers (*Amphiporeia virginiana*) (Marcogliese 1993*b*) from the beaches of Sable Island as well as in a gammarid (*Marinogammarus obtusatus*) (Val'ter 1987) and a caprellid (*Caprella septentrionalis*) (Val'ter 1978) from the White Sea.

Macroinvertebrate hosts of larval *P. decipiens* are not confined to the Amphipoda. Larval sealworm 2.69–8.96 mm in length have recently been identified in eastern Canadian mysids including *Neomysis americana* inhabiting the brackish waters of Wallace Lake, Sable Island (Marcogliese 1992*a*, 1993*a*), *Mysis stenolepis* from Passamaquoddy Bay, N.B. (Marcogliese and Burt 1993), and recently ingested *M. mixta* from stomachs of heavily infected American plaice caught near Sable Island (Martell 1992). Val'ter and Popova (1974) also describe a 3.72 mm *P.* (*Terranova*) *decipiens* larva in an errant polychaete, *Lepidonotus squamatus* (Annelida), from the White Sea, while in the laboratory, McClelland (1990) transmitted sealworm not only to polychaetes (juvenile *Phyllodoce* sp.) but also to sea slugs, *Coryphella* sp. (Mollusca: Gastropoda).

Helminth parasites of cold-ocean fishes often have low host specificities (Holmes 1990) and larval *P. decipiens*, which infects a broad spectrum of marine and euryhaline fishes in the North Atlantic and adjacent waters (McClelland et al. 1990), appears not to be an exception in this regard. Hence, it is not surprising that 12 species from 10 families and seven orders of fish were susceptible to larval sealworm infection in the present study (Table 1). Cod (McClelland et al. 1983*a*, 1983*b*, 1985; Brattey et al. 1990) and, to a lesser extent, smelt (Templeman et al. 1957; Landry and Hare 1990) appear to be the most important natural hosts of *P. decipiens* among fishes used in our experiments, however, the parasite also occurs naturally in alewife (Landry et al. 1992), pollock (McClelland et al. 1990), threespine stickleback (Marcogliese 1992*b*), and winter flounder (Scott 1954). Moreover, there are anecdotal reports of larval sealworm in brook trout (McClelland et al. 1990) and Atlantic salmon (Ramakrishna et al. 1993) and *Phocanema* sp. larvae identified in Newfoundland cunner (Sekhar and Threlfall 1970) may be referred to *P. decipiens* as well (Margolis and Arthur 1979). Records of natural sealworm infection are lacking for three laboratory hosts, Atlantic silverside, mummichog and grubby and, to our knowledge, neither silverside nor grubby have been surveyed for endoparasitic helminths.

Although the fact that larval *P. decipiens* levels in natural fish populations vary dramatically with host species (McClelland et al. 1990) seems indicative of some degree of host specificity, infection levels are not consistent with host phylogeny. Heavily infected fishes are phylogenetically diverse, being found in at least four orders of teleosts and, at the same time, belonging to families which also include lightly infected and uninfected species. Larval sealworm levels in natural fish populations are probably influenced by environmental or behavioural factors that may limit exposure to the parasite in nature but are suppressed in laboratory transmissions in which fish are confined to aquaria and hand-fed. Pollock and winter flounder, for example, became heavily infected in laboratory experiments in this study but are rarely infected in their natural environments, even when they occupy the same range as other heavily infected gadids and pleuronectids (McClelland et al. 1990). Whereas the early life history of sealworm is benthic and the larvae infect fish which either exploit benthos directly or prey upon smaller benthophagous fish (McClelland 1990), pollock are midwater consumers of nekton (Scott and Scott 1988) and would seldom be exposed to infection. Although they are benthic consumers, winter flounder feed on infauna or epifauna clinging to sessile organisms such as ectoprocts, while heavily infected flatfishes, e.g., American plaice, exploit more active benthic suprafauna including mysids and, perhaps, other important invertebrate hosts of sealworm (Martell and McClelland 1994).

Natural levels of sealworm infection in fish may also be influenced by the host response (McClelland et al. 1983*a*). In hosts where the nematodes are encapsulated and destroyed and the resulting lesions are ultimately replaced by normal tissue, the only sealworm larvae in evidence would be those surviving from recent reinfections. Infections in fish hosts which fail to mount such a response, on the other hand, might consist of sealworm larvae accumulated over the life span of the host. It follows that fishes with high levels of larval sealworm infection may include not only those species that are frequently exposed to the parasite, but also species in which the tissue response to infection is benign or lacking.

European (*Osmerus eperlanus*) and rainbow smelt are examples of fish hosts that seldom mount a tissue response to larval sealworm infection. An early histological study of host tissues surrounding larvae of *P.* (*Porrocaecum*) *decipiens* in the flesh of various fish species from European waters (Kahl 1938) reveals that while nematodes in larger hosts such as Atlantic cod, European ling (*Molva vulgaris*), and blue ling (*M. byrkelange*, syn. of *M. dypterigia*) were frequently enclosed in capsules of fibrous connective tissue, those infecting *O. eperlanus* from the Elbe River estuary were usually unencapsulated. In a more recent survey, Möller and Klatt (1990) report encapsulated *P. decipiens* larvae in only 2–3% of infected Elbe River smelt. The apparent absence of a tissue response to larval sealworm in experimentally infected *O. mordax* in the present study is consistent with Scott and Black's (1960) observation that *P.* (*Porrocaecum*) *decipiens* larvae in the flesh of rainbow smelt from the Bras d'Or Lakes, N.S. were seldom encapsulated. Scott and Black also noted that the tissue response to *P. decipiens*, frequently evident in mature cod from the Bras d'Or Lakes was rarely found in younger (≤5 years) cod. Hence, the tissue response to larval sealworm infection in fish may vary not only with host species but also with age or maturity of host.

While encapsulated sealworm larvae, many of which are dead and in various stages of degeneration, are often found in marine fish (Kahl 1938; Scott and Black 1960), the time frame for the development of the host tissue response and subsequent destruction of the nematodes is not known. According to Ramakrishna and Burt (1991), sealworm in the

musculature of experimentally infected rainbow trout (*Oncorhynchus mykiss*) were enclosed in capsules consisting of an inner layer of inflammatory cells and an outer layer of fibroblasts laying down collagen by 16 days PE at 15°C. By 32 days, capsules had become more fibrous and compact and the inner inflammatory layer had begun to degenerate. In the present study, fibrous capsules enclosed all sealworm larvae ($n = 87$) in two experimentally infected grubby and 37 of 42 *P. decipiens* in a winter flounder after 56 days at 15° C. Although the five unencapsulated nematodes in the flounder were viable and had apparently undergone significant growth, all encapsulated nematodes in grubby and flounder were moribund.

Widespread deposition of fat in the body cavities and adjacent musculature of our experimentally infected mummichog seemed to resemble lipoid degeneration such as that which occurs in farmed fish as a consequence of nutritional deficiencies or imbalances in the diet (Schäperclaus 1992). Although not a typical response to parasitic infection, the condition was not observed in unexposed controls and thus did not seem to be merely coincidental to the sealworm infection. Moreover, nematodes entrapped in fatty connective tissue usually died; 57 (40%) of 144 encapsulated *P. decipiens* were moribund after 28 days in mummichog and by 56 days, 100% of the encapsulated nematodes had expired. Viable, unencapsulated nematodes were largely confined to the musculature and connective tissues of the head and tail, tissues that were not impregnated with fat.

Evidently, larval sealworm infections can be lethal to small fish. Möller et al. (1991) report that 4 of 22 small (22–24 cm), cultured European eel (*Anguilla anguilla*) which were force-fed larval *P. decipiens*, died within 24 h of exposure. In the present study, 15 mummichog and a grubby died 10–20 days after incurring experimental sealworm infections. Given that natural fish hosts of larval *P. decipiens* may be as small as those used in the experiments above and that sealworm levels in natural hosts may rival or exceed those achieved in laboratory transmissions, it is possible that the parasite may also cause mortality in free-living fish. Threespine stickleback, the smallest experimental host used here, is naturally infected in brackish ponds on Sable Island (Marcogliese 1992*b*); *P. decipiens* has been found in Sable Island stickleback weighing as little as 0.5 g (D.J. Marcogliese, Maurice Lamontagne Institute, Mont-Joli, Québec, personal communication). Current studies of small benthophagous fishes from waters surrounding Sable Island (McClelland 1990; G. McClelland, unpublished data) indicate that Atlantic hookear (*Artediellus atlanticus*) and moustache sculpin (*Triglops murrayi*) and alligatorfish (*Aspidophoroides monopterygius*) weighing <2 g are often infected and that densities of the parasite in these three species, as well as in American plaice, may approach or, in some individual hosts, exceed one nematode per gram of body weight.

Death of experimental hosts in the present study seems to have been attributable to damage inflicted upon various vital organs and tissues by feeding and migrating sealworm larvae, but the parasite may contribute to fish host mortality by more subtle means. Volatile ketones that larval sealworm produce as metabolic byproducts may act as local anaethetics in surrounding host musculature (Ackman and Gjelstad 1975). This mechanism may have been a factor, for example, in experiments conducted by Sprengel and Lüchtenberg (1991) wherein the maximum swimming speed of European smelt declined with increasing intensity of larval *P. decipiens* infection. In densities such as those found in naturally infected fish above, the parasite could cause the host to become sluggish, impairing its ability to forage or avoid predators.

Larval sealworm described from free-living marine macroinvertebrates vary from 1.1 to 9.0 mm in length (McClelland 1990; Marcogliese 1992*a*, 1993*a*, 1993*b*; Martell 1992; Marcogliese and Burt 1993), large enough, as present results show, to infect various fish species. Because of the crude examination procedures routinely used in parasitological surveys of marine fish, however, *P. decipiens* larvae of this size are seldom reported from naturally infected hosts. In early surveys of cod and other groundfish species in eastern Canada (Scott and Martin 1957; Templeman et al. 1957), for example, the smallest nematodes detected by candling sliced or whole fillets with the unaided eye were 14–15 mm in length. Larvae <10 mm in length are usually apparent to the naked eye on visceral organs and mesenteries (McClelland et al. 1983a) but difficult to discern from muscle fibres in groundfish fillets. On the other hand, Möller and Klatt (1990) found *P. decipiens* larvae as small as 6.7 mm in length by candling whole juvenile smelt from the Elbe River, while Pálsson (1979) detected 4–5 mm sealworm larvae in juvenile Icelandic cod by microscopic examinations of tissue squashes. In our own recent surveys (G. McClelland, unpublished data) pepsin–HCl digestions have been employed in the recovery of sealworm larvae as small as 3 mm in length from small benthophagous fish.

Larval sealworm similar in size to those found in benthic invertebrates would occur most commonly in small fish that feed directly on benthos. Large, commercially important fish like mature cod are often piscivorous and would tend to accumulate nematodes that have, perhaps, already undergone substantial growth and development in smaller prey fish. As revealed by present results, small sealworm larvae may have difficulty establishing themselves in large fish hosts because of the greater scale of the migration required to reach the skeletal musculature. After 24 h *P. decipiens* larvae had reached the flesh of smaller hosts such as smelt and mummichog but were still confined to the food bolus in the stomach of cod. After 7 days in cod, only 12% of sealworm larvae occurred in musculature and 35% of the nematodes had not completed penetration of the gut wall. In transmissions to mummichog, larger nematodes were more successful in migrating to the skeletal musculature where they subsequently were able to avoid the host tissue response. Early migration to the flesh of mummichog seemed critical to the survival of the nematode, as the proportion of nematodes in the flesh did not change after 24 h and virtually all larvae remaining in the body cavity beyond this time were eventually encapsulated and destroyed.

Field studies have provided us with little or no insight into the growth and development of larval sealworm in natural fish hosts. Möller and Klatt (1990) note that mean length of larval *P. decipiens* in Elbe River smelt increased

significantly with host age, varying from 23 mm in 0-group fish to 33 mm in 4 year olds. While they may be indicative of sealworm growth, nematode length increments in successive smelt year classes are also open to other interpretations, e.g., as the fish mature consuming progressively larger prey, they may also be exposed to and reinfected by larger nematodes. Present experimental results, however, show that larval *P. decipiens* may undergo substantial growth in fish hosts. Growth in sealworm body length, initially exponential and subsequently becoming asymptotic in crustacean hosts (McClelland 1982, 1990), resumed at a linear rate in experimentally infected fish. Present results also show that the growth rate of the larvae varied with host species, being significantly more rapid in smelt than in mummichog. Sealworm larvae surviving in mummichog, in fact, grew no faster than those maintained in vitro. The retarded growth of sealworm larvae escaping the tissue reaction in mummichog and winter flounder may have been a further manifestation of the host response.

The first two moults (M_1 and M_2) in the life cycles of a number of ascaridoid species are reported to occur in the egg (Anderson 1992) and Køie (1993) has proposed that prehatch development to L_3 is typical of most anisakids, including *P. decipiens* (Køie and Fagerholm 1993). Although only one moult has been detected in the egg of *P. decipiens*, there is considerable indirect evidence that this species hatches to the L_3. According to an in vitro investigation (McClelland and Ronald 1974), larvae that emerge from the eggs of *P. decipiens* and subsequently escape from their sheathes (L_2 cuticles ?) grow directly to the infective stage without performing a moult. Further, experimental transmissions to seals (McClelland 1980) revealed that the final two moults (M_3 and M_4) in the sealworm life cycle take place in the stomach of the definitive host and, hence, that the infective stage is the L_3. Finally, in studies of the in vivo development of the parasite in experimental crustacean (McClelland 1982, 1990) and fish hosts (this study), neither moulting nematodes nor cast cuticles were found. Also lacking was indirect evidence of sealworm moults such as changes in nematode morphometry similar to those associated with M_3 and M_4 in seals (McClelland 1980). Despite the abrupt change in the growth rate of the parasite following transmissions to fish in the present study, *P. decipiens* from fish were morphometrically consistent with sealworm of similar size from amphipods.

Acknowledgments

The author is extremely grateful to D.J. Martell, Department of Fisheries and Oceans, Halifax Fisheries Research Laboratory, for assisting in the analyses and in the preparation of the manuscript. The laboratory work was performed in the Biology Department, Dalhousie University, and was supported, in part, by United States Department of Health, Education and Welfare contracts 223-75-2127 and 223-77-2128.

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The number of moults in the egg of sealworm, *Pseudoterranova decipiens* (Nematoda: Ascaridoidea): an ultrastructural study

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Abstract: The number of moults in the egg of sealworm, *Pseudoterranova decipiens* (Nematoda: Ascaridoidea), and other ascaridoids is contentious. Transmission electron microscopic analysis of eggs and free-living larvae of sealworm confirmed that only one moult occurs in the egg. The first-stage larval (L_1) cuticle on embryos was first observed in eggs incubated at 15°C in sea water on day 5 after eggs were dissected from the uterus of sealworm obtained from the stomach of grey seals. There was no ecdysis of this L_1 cuticle. A second cuticle began to form beneath the L_1 cuticle between day 5 and 12. The second-stage larval (L_2) cuticle continued to develop and on day 12 the L_2 larva hatched enclosed within the cuticle of the first stage. The L_1 cuticle appeared to be partially resorbed during development of the L_2 cuticle. This study provides the first ultrastructural evidence of the number of moults that occur in eggs of sealworm.

Résumé : Le nombre de mues dans l'oeuf du ver de phoque, *Pseudoterranova decipiens* (Nematoda : Ascaridoidea) et d'autres ascaridoïdes est controversé. L'analyse d'oeufs et de larves libres de ver de phoque par microscopie électronique à transmission a confirmé qu'il se produit seulement une mue dans l'oeuf. La cuticule du premier stade larvaire (L_1) sur les embryons a été observée pour la première fois dans des oeufs incubés en eau de mer à 15°C le 5^e jour après le prélèvement des oeufs dans l'utérus de vers de phoque recueillis dans l'estomac de phoques gris. Il n'y a pas eu d'ecdysis de cette cuticule L_1. Une seconde cuticule a commencé à se former sous la cuticule L_1 entre le 5^e et le 12^e jour. La cuticule du second state larvailre (L_2) a continué à se développer et, le 12^e jour, la larve L_2 a éclos, enfermée dans la cutivule du premier stade. La cuticule L_1 semble s'être partiellement résorbée pendant le développement de la cuticule L_2. Cette étude présente la première preuve ultrastructurale sur le nombre de mues qui se produisent dans l'oeuf du ver de phoque.

[Traduit par la Rédaction]

Since 1945 Canadian parasitologists and fishery research scientists have been studying the life history of sealworm, *Pseudoterranova decipiens* (see Scott 1953; Bowen 1990). These 50 years of research have contributed greatly to our understanding of the biology and epizootiology of this economically important parasite. Early studies on the development of sealworm stated that only one moult occurs in the egg and that it is the second-stage larva ensheathed with the cuticle of the first stage that hatches from the egg (Scott 1955; Myers 1960; McClelland and Ronald 1970, 1974). These early studies were supported by more extensive work on post-embryonic development and experimental studies involving invertebrates, fish, and marine mammals (McClelland 1980*a*, 1980*b*, 1980*c*, 1982, 1990); however, evidence has not been presented at the ultrastructural level to confirm light microscopic findings. Ecdysis of the first-stage cuticle occurs in various invertebrates (McClelland 1982, 1990). It was determined that the third and fourth moult occur in the definitive host, the seal (McClelland 1980a). The location of the second moult, however, has not been detected.

Some ascaridoids are reported to moult twice in the egg (Thust 1968; Araujo 1971, 1972; Maung 1978; Araujo and Machado 1980; Berry and Cannon 1981; Prociv 1989; Koie 1993; Koie and Fagerholm 1993). Some of these and other observations on moulting in ascaridoid eggs are inconsistent with results obtained experimentally with intermediate and definitive hosts, which has led to conflicting conclusions on the location of the four moults. This study was undertaken to determine if previous light microscopic findings on the number of moults occurring in the egg of *Pseudoterranova decipiens* are accurate. Observations made at the light microscopic level of intact and broken eggs of sealworm are also presented.

Received April 11, 1993. Accepted May 20, 1994.
J12522

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Materials and methods

Live mature sealworms, *Pseudoterranova decipiens*, were obtained from two grey seals, *Halichoerus grypus*, from the Northumberland Strait near Port Hood, Nova Scotia (46°01′N, 61°32′W). Worms were maintained in mammalian saline (0.85% NaCl) at 0°C for 4 d. Females were dissected and small pieces (3–6 mm) of uterus containing shelled eggs were transferred to each of 15 Petri dishes containing 35‰ sea water. Dishes containing uteri were incubated at 15°C and development of eggs was checked daily. Light microscopic study was conducted by removing eggs and larvae of sealworm developing at 15°C in sea water (35‰), fixing in hot glycerin–alcohol (1 part glycerin: 9 parts 70% alcohol) and clearing by allowing the alcohol to evaporate. Eggs in glycerin on glass slides were broken with light glass coverslip pressure.

Uteri containing developing eggs were fixed for transmission electron microscopy at days 0, 1, 2, 3, 3.5, 4.5, 5, 5.5, 6, 7, 9, 10, 11, 12, and 15 using 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.2) for 2 months at 5°C, post-fixed with 1.0% osmium tetroxide in 0.15 M phosphate buffer for 1 h at room temperature, dehydrated through an ascending ethanol series, infiltrated and embedded in Spurr's resin (Pelco International, Redding, Calif.). Ultrathin sections, cut with a diamond knife on a Reichert Ultracut E, were stained with uranyl acetate in 50% methanol, post-stained with Reynold's lead citrate, and examined using a Hitachi H7000 transmission electron microscope operated at 75 kV.

Voucher specimens of adult *Pseudoterranova decipiens* are deposited in the Canadian Museum of Nature, Ottawa, Ont., as collection number CMNP-1994-0055.

Results

Light microscopy

Eggs removed from the distal extremity of uteri of gravid adult sealworms possessed a shell and contained an embryo in the morula stage with 8–16 cells (day 0). After 1 d incubation at 15°C eggs were in the gastrula stage (day 1). On day 2, eggs contained early tadpole stages and on day 4.5, late tadpole stages. Some eggs on day 5 contained first-stage larvae, each with a boring tooth and one cuticle. Second-stage larvae, each with a boring tooth and two cuticles (the cuticle of the second-stage larva and the retained cuticle of the first stage), developed in eggs from days 5 to 12. Hatching began at day 11 and by day 15, almost 100% of larvae had hatched.

Hatched second-stage larvae retained the cuticle of the first stage. Hatched second-stage larvae possessed a boring tooth and resembled those described by McClelland (1982).

Eggs were broken manually with light coverslip pressure to observe developing larvae and any cuticle shed within the egg. Occasionally the first-stage cuticle of larvae in eggs on day 7 was deformed during breakage of the eggshell and ballooned out in the cephalic region. No remnants of cuticle shed in the egg were observed. Spontaneously hatched larvae which remained attached to eggshells from which they exited had two cuticles, the outer one being loose and retained as a sheath. No shed cuticle was visible in or near empty eggshells.

Electron microscopy

The eggshell consisted of three distinct layers: outermost, a thin electron-dense unit-membrane-like layer; middle, a thick electron-lucent layer; and innermost, an electron-dense layer, loosely attached to the middle layer, of variable width and form (Fig. 1). On some sectioned eggs irregular strands of particulate material projected outward from the outermost layer (Fig. 2).

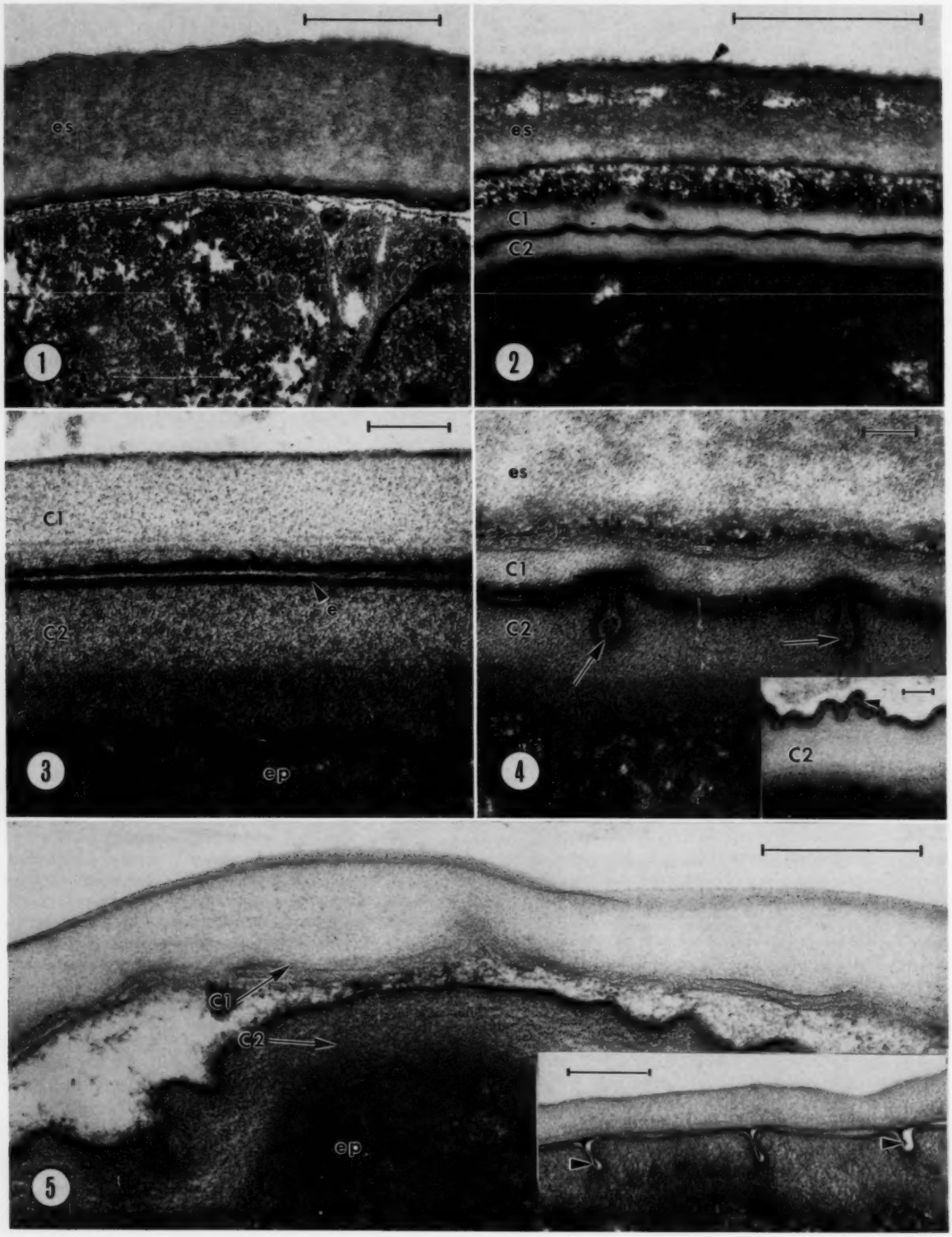
All eggs sectioned on days 5 and 7 contained embryos still in the tadpole stage and no first-stage cuticle was observed (Fig. 1). On day 9 most sectioned eggs contained larvae with two cuticles, the outer first- and the inner second-stage cuticle (Fig. 2), separated by electron-dense granular material (Figs. 2, 3). On day 9 the cuticle of the first stage (0.14–0.16 μm) was similar in thickness to that of the second stage (0.12–0.15 μm). By day 12, however, the cuticle of the unhatched second stage had thickened (1.8–2.0 μm) (Fig. 3). The cuticle of the first-stage larva appeared almost entirely electron-lucent, while the cuticle of the second stage had an outermost electron-lucent and an innermost electron-dense layer just above the epidermis (see Fig. 3). Electron-dense granular material occurred between the two cuticles (Figs. 2–4). The newly forming cuticle of the second stage was convoluted or folded with electron-dense granular material above and below the folds and with similar material above the convoluted epidermis (Fig. 4). Second-stage larvae that hatched from eggs on day 12 retained the cuticle of the first stage. The new cuticle of the hatched second stage on day 12 was thicker (3.7–4.8 μm) than that of unhatched second-stage larvae on day 11 (2.0–2.6 μm) (Figs. 4, 5). In some sections of hatched larvae, electron-dense granular material projected from the inner surface of the old cuticle into depressions or small infoldings (annuli) of the new second-stage cuticle (Fig. 5). At no point during development was a shed cuticle seen within the egg.

Discussion

This study shows that only one moult occurs in the egg of sealworm and that the second-stage larva, within the cuticle of the first stage, hatches from the egg. This confirms previous studies using light microscopy (Scott 1955; Myers 1960; McClelland and Ronald 1970, 1974). The time of development of sealworm eggs incubated in sea water at 15°C is also similar to that reported by Scott (1955), Myers (1960), McClelland and Ronald (1970, 1974), McClelland (1982), and Burt et al. (1990).

While eggs of sealworm do not have an uterine layer, as reported in some ascaridoids (Foor 1967; Wharton 1979, 1980), they are typical of most nematodes in that they have a vitelline membrane (outermost layer of eggshell), a chitinous layer (middle layer of eggshell) and a lipid layer (innermost layer of eggshell) (see Bird and Bird 1991). The resistant nature of the eggshell to some fixatives may have impeded early studies of sealworm eggs. The strands of particulate material evident on the vitelline membrane in the present study were observed by Wharton (1979) in *Porrocaecum ensicaudatum* and in other nematodes (see Bird and Bird 1991).

In this study, at the electron microscopic level the first-stage cuticle was observed only in association with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Figs. 1–5. Electron micrographs of eggs and larvae of sealworm, *Pseudoterranova decipiens*. Fig. 1. Embryo in egg on day 5. Note eggshell (*es*) and absence of cuticle. Scale bar = 1 μm. Fig. 2. First-stage larva in egg on day 9. Note first-stage cuticle (C1), forming second-stage cuticle (C2), eggshell (*es*), and vitelline membrane (arrowhead). Note irregular strands of particulate material projecting outward from vitelline membrane. Scale bar = 1 μm. Fig. 3. Second-stage larva in egg on Day 12. Note first- and second-stage cuticles. Also note epicuticle (*e*) and epidermis (*ep*) of second-stage larva. Scale bar = 0.1 μm. Fig. 4. Second-stage larva in egg on day 11. Note folds (arrows) on newly forming second-stage cuticle. Scale bar = 0.1 μm. Inset shows electron-dense material (arrowhead) of second-stage cuticle. Scale bar = 0.1 μm. Fig. 5. Second-stage larva naturally hatched from egg on day 12. Note retained first-stage cuticle (C1), second-stage cuticle (C2), and epidermis (*ep*). Section is cut at an oblique angle. Scale bar = 0.5 μm. Inset shows electron-dense material (arrowheads) between first- and second-stage cuticles. Scale bar = 0.5 μm.

a developing second-stage cuticle. In no sections cut prior to day 9 (no eggs were fixed at day 8) was only one cuticle (i.e., that of the first stage) seen. This suggests that the second-stage cuticle begins to form very quickly after the first-stage cuticle is formed (probably within hours). The electron-dense material observed between the first- and second-stage cuticles, near convoluted sections of cuticle and adjacent to the epidermis of the second-stage larva, seems indicative of intense biosynthetic activity associated with moulting or cuticle formation (see Bird and Bird 1991). The cuticle of nematodes has various layers or zones, from the outer epicuticle to the cortical, median, and innermost basal zones (see Bird and Bird 1991). The intense activity as well as the apparent absence of the electron-dense basal zone in the first-stage cuticle suggests that the first-stage cuticle is partially resorbed. Resorption of the first-stage cuticle is particularly evident near convoluted areas (annuli) of the new second-stage cuticle. In addition, the sheath (first-stage cuticle) enclosing hatched second-stage larvae appears to be comprised of the cortical zone only. Resorption continues for an indefinite period after larvae hatch. Partial resorption of the shed cuticle of *Ascaris lumbricoides* larvae developing in mice has been reported by Thust (1968).

In zooparasitic nematodes, the third and fourth moults usually occur in the definitive host. In *Pseudoterranova decipiens* the third and fourth moult have been observed in seals (McClelland 1980a). As the first moult (apolysis and production of the second cuticle) occurs in the egg of sealworm and ecdysis (shedding) of the first-stage cuticle occurs in invertebrates (McClelland 1982, 1990) the location of the second moult has yet to be determined. Various studies with invertebrate and fish hosts have yet to identify the location of this moult (McClelland 1980*a*,1980*b*, 1980*c*, 1982, 1990). Based on the findings of this study, further efforts should now be concentrated on development in invertebrates.

Ascaridoids reported to moult twice in the egg include *Contracaecum osculatum*, *Hysterothylacium aduncum*, *Sulcascaris sulcata*, *Ascaris suum*, *A. lumbricoides*, *Hexametra boddaertii*, *Ophidascaris sprenti*, *Travassoascaris araujoi*, *Ophidascaris trichuriformis*, *Toxocara pteropodis*, *T. cati*, and *T. canis* (see Thust 1968; Araujo 1971, 1972; Maung 1978; Araujo and Machado 1980; Berry and Cannon 1981; Prociv 1989; Koie 1993; Koie and Fagerholm 1993). Most studies reporting two moults in the egg have been based on examination of larvae forced from developing eggs using coverslip pressure. Artificial eclosion may cause artifacts as seen in this study (also see Schacher 1957; Prociv 1989) and results should be interpreted with caution. For example, layers of the cuticle may separate with pressure and one cuticle may appear as two cuticles.

Reports of two moults in the eggs of ascaridoids are inconsistent with reports of three moults observed in the intermediate and definitive hosts of at least seven species of ascaridoids (i.e., *Raphidascaris acus*, *Ascaris suum*, *Baylisascaris devosi*, *Toxoascaris leonina*, *Porrocaecum ensicaudatum*, *Toxocara canis*, and *Amphicaecum robertsi* (see Sprent 1953, 1959, 1963*a*, 1963*b*; Kelly et al. 1957; Douvres et al. 1969; Smith 1984; McNeill and Anderson 1990*a*, 1990*b*; also see Anderson 1992 for a review).

As Douvres et al. (1969) observed in *Ascaris suum* the process of ecdysis may occur outside the egg within a host and this may complicate interpretation of results from experimental hosts. It is premature to generalize that other members of Ascaridoidea, including the Anisakidae, moult twice within the egg (Koie 1993, Koie and Fagerholm 1993). The location and timing of moults in ascaridoids are likely species specific and are probably adaptations to the anatomy, physiology, and behaviour of their hosts, all of which can influence reproductive success and subsequent transmission. Clearly, ultrastructural studies of eggs and larvae in concert with careful experimental work to locate all four moults of all species of ascaridoids in their respective hosts are needed to resolve contentious findings and to understand better the dynamics of transmission.

Acknowledgments

We thank Dr. M.O. Hammill who facilitated collection of seals. Pete Marcott and Pierre Rivard assisted in the field and laboratory. We also thank Drs. G. McClelland, S.S. Desser, and R.C. Anderson for their comments on the manuscript. A portion of this research was funded by the Natural Sciences and Engineering Research Council of Canada (research grant 6965 to Dr. S.S. Desser).

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Philometra margolisi n.sp. (Nematoda: Philometridae) from the gonads of the red grouper, *Epinephelus morio* (Pisces: Serranidae), in Mexico

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Abstract: A new nematode species, ***Philometra margolisi*** n.sp., is described from the gonads of the serranid fish *Epinephelus morio* from Mexico (localities Telchac, Chuburna, Progreso, and Rio Lagartos, Yucatan). It is characterized mainly by long (0.432–0.468 mm) and slender spicules and a simple gubernaculum (length 0.084–0.093 mm) in the male and by body shape and size (length 65–85 mm), arrangement and number of small cephalic papillae (eight papillae in an outer circle and four in an inner one), structure and length (1.07–1.18 mm) of the esophagus, long ovaries, and the presence of a pair of small lateral papilla-like projections on the elongate caudal end in gravid females.

Résumé : Le présent article vise à décrire une nouvelle espèce de nématode, ***Philometra margolisi*** n.sp., isolée dans les gonades du serranidé *Epinephelus morio*, du Mexique (Telchac, Chuburna, Progreso et Rio Lagartos, au Yucatan). Le mâle est pourvu de spicules longs (0,432–0,468 mm) et étroits et d'un gubernaculum simple (longueur : 0,084–0,093 mm), tandis que la femelle gravide se caractérise par la forme et la taille du corps (65–85 mm), la disposition et le nombre de petites papilles céphaliques (couronne externe de huit papilles encerclant une couronne interne de quatre papilles), la structure et la longueur (1,07–1,18 mm) de l'oesophage, des ovaires étendus et la présence sur l'extrémité apicale de la caudale allongée d'une paire de petites projections papilliformes.

[Traduit par la Rédaction]

Introduction

The red grouper, *Epinephelus morio* (Serranidae), is one of the principal fishery resources of coastal Yucatan and Cuba. At least three fleets depend on the exploitation of this fish: the artisanal and industrial fleets of Yucatan, Mexico, and the industrial fleet of Cuba (Solana-Sansores and Arrequin-Sanchez 1991). The importance of this resource has given rise to an increase in research on its biology by Mexican and Cuban institutions in the last few years.

During preliminary studies of the helminth fauna of *E. morio* carried out by the Mexican co-authors, a new nematode of the genus *Philometra* Costa, 1845 was found parasitizing the ovary. The aim of this paper is to describe it and to present some data on the level of infection by *Philometra margolisi* n.sp. in *E. morio* for different localities along the Yucatan Peninsula.

Received August 4, 1994. Accepted November 22, 1994. J12489

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Materials and methods

Nematodes were fixed in hot 4% formaldehyde in saline and stored in 70% ethanol. "En face" views were prepared according to Anderson's (1958) method. For examination, the nematodes were cleared in glycerine. Drawings were prepared with the aid of a Zeiss microscope drawing attachment. After examination, some specimens were embedded in glycerine–gelatin as permanent mounts; remaining specimens are stored in 70% ethanol. Measurements, presented as the range followed by the mean ±SD in parentheses, are given in millimetres.

***Philometra margolisi* n.sp.** Fig. 1

Male (10 specimens; measurements of holotype in parentheses)

Body thread-like, whitish, with smooth cuticle; length 1.95–2.44 (2.44) [2.19 ± 0.25], maximum width 0.041–0.054 (0.054) [0.048 ± 0.007]. Cephalic end dome shaped. Mouth

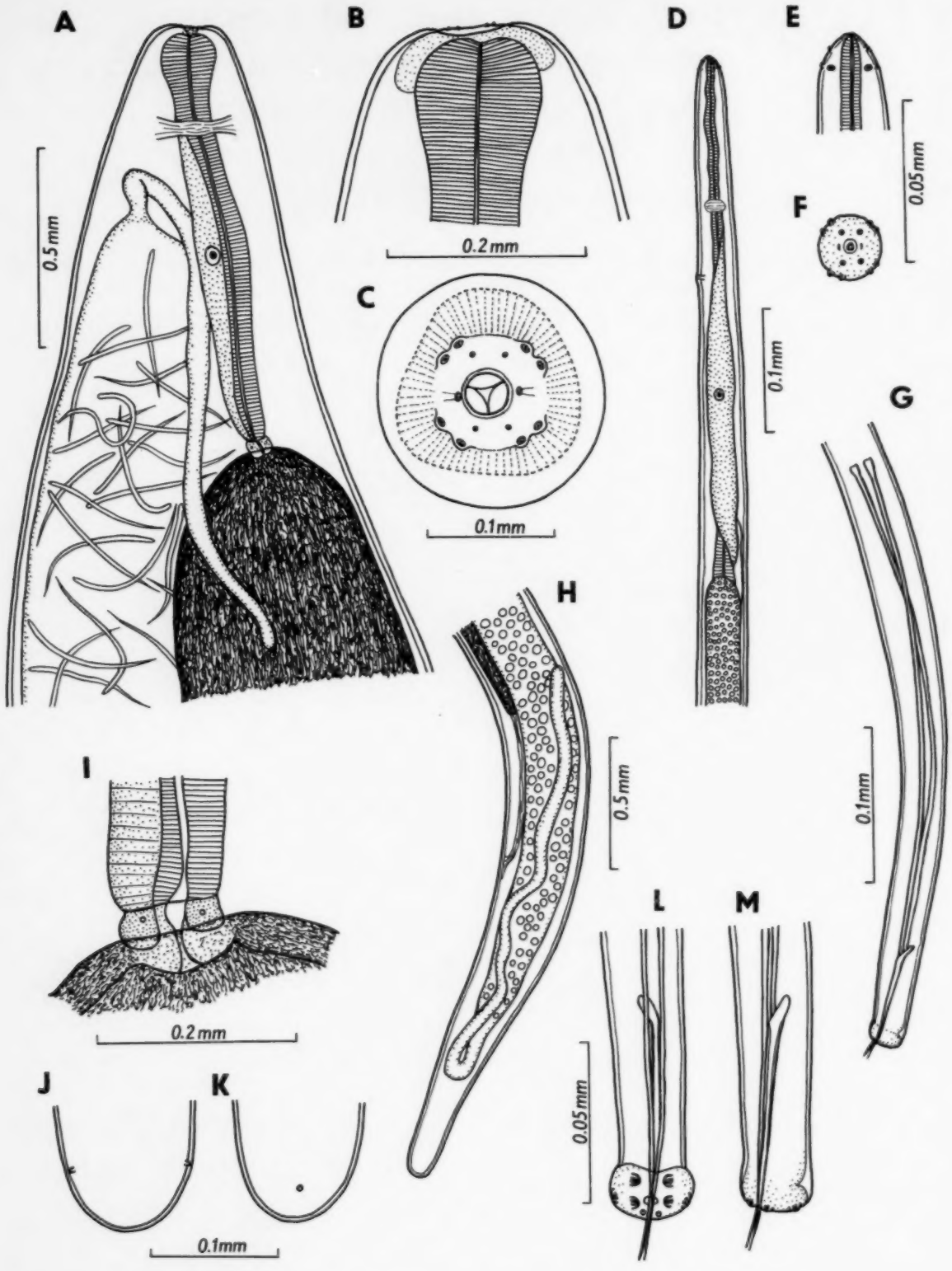
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Fig. 1. *Philometra margolisi* n.sp. (A) Anterior end of gravid female. (B, C) Cephalic end of gravid female, lateral and apical views. (D) Anterior end of male. (E, F) Cephalic end of male, lateral and apical views. (G) Posterior end of male. (H) Posterior end of young female. (I) Esophageo-intestinal junction of female. (J, K) Caudal extremity of female, dorsoventral and lateral views. (L, M) Caudal end of male, ventral and lateral views.

Table 1. Prevalence and abundance of *Philometra margolisi* n.sp. in *Epinephelus morio* from the coast of Yucatan Peninsula, Mexico.[a]

Locality	No. of fish examined	Fish standard length (cm)		Parasite		
				Prevalence (%)	Abundance	
		$\bar{x} \pm SE$	Range		$\bar{x} \pm SE$	Range
Chuburna	7	23.2±1.4	19.7±30.6	28.6	1.3±0.8	0–5
Progreso	11	23.3±1.4	16.1±27.4	27.3	1.5±0.8	0–8
Telchac	7	26.3±4.8	10.0±48.5	28.6	4.0±2.6	0–15
Rio Lagartos	7	23.7±4.0	9.0±37.0	14.3	0.1±0.0	0–1

[a]Prevalence and abundance are used as defined in Margolis et al. (1982).

very small, circular, surrounded by two circles of minute papillae and two lateral amphids; outer circle of papillae formed by four pairs of dorsoventral and ventrolateral papillae, inner circle by four single papillae. Esophagus narrow, without inflation at its anterior end, its overall length 0.450–0.531 (0.453) [0.491 ± 0.041]; posterior part of muscular esophagus overlapped by well-developed dorsal esophageal gland with large cell nucleus. Esophageal nucleus 0.294–0.315 (0.303) [0.305 ± 0.011], nerve ring 0.114–0.138 (0.138) [0.126 ± 0.012], and excretory pore 0.168–0.204 (0.204) [0.186 ± 0.018] from anterior extremity, respectively. Posterior end of body distinctly lobed, with one pair of preanal and two pairs of postanal flat papillae; outlets of phasmids located subterminally on posterior body end. Spicules very narrow, needle-like, equally long, with somewhat expanded proximal tips; length of spicules 0.432–0.468 (0.432) [0.450 ± 0.018], their width at middle 0.002 (0.002) [0.002 ± 0.000], at proximal ends 0.006 (0.006) [0.006 ± 0.000]. Gubernaculum narrow, 0.084–0.093 (0.087) [0.087 ± 0.005] long, its distal tip sharply pointed, simple, without usual reflected dorsal barb; its proximal tip dorsally bent, less sclerotized than the remainder. Spicules and gubernaculum well sclerotized, yellow-brown in color. Length ratio of gubernaculum and spicules 1:5.0–5.5 (1:5.0).

Gravid female with larvae (eight specimens; measurements of allotype in parentheses)

Body of fixed female brownish in color, tapering slightly to both ends; posterior part of body distinctly narrower than anterior. Cuticle smooth. Length of body 65–85 (65) [75 ± 10] maximum width 0.938–1.183 (1.061) [0.980 ± 0.218], width near posterior end 0.408–0.544 (0.408) [0.476 ± 0.068]. Anterior end of body rounded. Oral opening circular, surrounded by two circles of small cephalic papillae and pair of lateral amphids; their number and position similar to those of male. Esophagus narrow, swollen near mouth to form distinct bulb 0.122–0.150 (0.122) [0.136 ± 0.014] long and 0.136–0.165 (0.150) [0.152 ± 0.30] wide. Overall length of esophagus 1.07–1.18 (1.09) [1.12 ± 0.05], representing 1.4–1.6% (1.6) of body length. Dorsal esophageal gland prominent, extending anteriorly to level of nerve ring and posteriorly to small ventriculus. Esophageal gland provided with giant cell nucleus located in about its middle, 0.598–0.734 (0.707) [0.666 ± 0.068] from anterior extremity. Nerve ring 0.231–0.286 (0.258) [0.259 ± 0.028] from anterior extremity. Ventriculus 0.024–0.068 (0.068) [0.046 ± 0.022] long, 0.075–0.105 (0.095) [0.090 ± 0.015] wide. Intestine brown colored, straight, its posterior end atrophied, forming ligament attaching ventrally to body wall, far anterior to posterior extremity; width of intestine slightly behind ventriculus 0.462–0.734 (0.544) [0.598 ± 0.136]. Posterior end of body rounded, bearing two small, hardly visible lateral papilla-like projections. Vagina and vulva absent. Ovaries long, narrow, situated near anterior and posterior ends of body; anterior ovary reaching to about mid-length of esophagus, sometimes up to level of nerve ring, posterior ovary far behind intestinal ligament, sometimes nearly extending to posterior end of body. Uterus occupying most of body, filled with eggs, developing embryos and first-stage larvae; length of larvae ($n = 10$) 0.420–0.444 [0.432 ± 0.012], maximum width 0.012 [0.012 ± 0.000].

Measurements of one female with eggs: Body length 35, width 0.694; esophagus 0.90 long, representing 2.6% of body length. Nucleus of esophageal gland and nerve ring 0.571 and 0.245, respectively, from anterior extremity. Ventriculus measuring 0.041 × 0.082.

Type host: Red grouper, *Epinephelus morio* (Perciformes, Serranidae).

Location: Gonads.

Type locality: Telchac (21°20′N, 89°07′W) (collected 27 June 1992); other localities: Progreso, Chuburna, and Rio Lagartos, all Yucatan, Mexico.

Etymology: This species is named in honor of Dr. Leo Margolis, a well-known Canadian parasitologist who has contributed greatly to the knowledge of fish nematodes and other helminth parasites.

Deposition of types: Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice (holotype, allotype and paratypes, Coll. No. N-622).

Occurrence: This nematode is a common parasite of *E. morio* in the localities studied; some parameters of infection are given in Table 1.

Discussion

According to the key to genera of the Philometridae provided by Moravec and Shaharom-Harrison (1989), these nematodes belong to the genus *Philometra* Costa, 1845 sensu stricto, which includes a large number of species parasitic in both freshwater and marine fishes. Most species are known only from females, whereas very small males have hitherto been described for only 16 species. It is important to note that females of some closely related species are indistinguishable morphologically and these species can be reliably identified only from male specimens.

The morphology of the female of *P. margolisi* n.sp. is very similar to that of *Philometra lateolabracis* (Yamaguti, 1935), as described by Yamaguti (1935), Rasheed 1963, Moravec et al. (1988), and others. Differences exist only in the length and shape of the caudal end of gravid females, which in *P. margolisi* is more slender and the intestine ends much more anteriorly, and the extent of the esophageal gland, which reaches posteriorly to the end of the esophagus in *P. margolisi*, but not in *P. lateolabracis*. The body length of gravid females of *P. lateolabracis* is usually longer (exceeding 100 mm) than that of *P. margolisi* (see, e.g., Yamaguti 1935, Rasheed 1963, Martínez and Ventosa 1982; Sakaguchi et al. 1987, Moravec et al. 1988).

Philometra lateolabracis was originally described by Yamaguti (1935) from females found in the ovary of the marine perciform fishes *Lateolabrax japonicus* (type host), *Parapristipoma trilineatum* and *Ephinephelus akaara* in Japan, from where it was also reported by Sakaguchi et al. (1987). Rasheed (1963) considered as synonymous with *P. lateolabracis* the species *P. scomberomori* (Yamaguti, 1935) and *P. sciaenae* Yamaguti, 1941, described from *Scomberomorus chinensis* and *Sciaena schlegeli*, respectively, from Japan. *Philometra lateolabracis* is now considered to be widespread mainly in the tropical regions of the Pacific, Indian, and Atlantic Oceans, being common in a variety of fishes (Yamaguti 1935, 1941; Rasheed 1963, 1965; Schmidt and Kuntz 1969; Rees 1970; Crisp and Klein 1973; Parukhin 1976; Martínez and Ventosa 1982; Sakaguchi et al. 1987; Moravec et al. 1988). However, nearly all these records have been based on findings of female specimens and it cannot, therefore, be excluded that several species have been confused under this name.

The only record of the male of *P. lateolabracis* is that of Crisp and Klein (1973), who described a single specimen found in the white grunt, *Haemulon plumieri*, from the Atlantic Ocean near the coast of Brazil. Although the description is inadequate, it shows clearly the length of the two subequal, slender spicules to be approximately 0.16 mm. In contrast, the spicules of *P. margolisi* are approximately three times longer, whereas the male body is distinctly shorter. The very long spicules of *P. margolisi* is a characteristic feature of this nematode. Of the species in which males have been described, only *P. katsuwoni* Petter and Baudin-Laurencin, 1986 from tunas of the Gulf of Guinea has spicules longer (0.74–1.25 mm) than those of *P. margolisi*, but the body of males is much longer (length 9.5–12 mm vs. 2–2.4 mm) (see Petter and Baudin-Laurencin 1986). In all other species the spicules are shorter than 0.2 mm, except in *P. ovata* (Zeder, 1803) from European freshwater cyprinids, where they attain a length of up to 0.36 mm (Moravec 1971). However, the gubernaculum of *P. ovata*, similar to that of some other *Philometra* species, is provided with a distinct reflected dorsal barb which is lacking in *P. margolisi*.

In most species of philometrids, conspecific males and gravid females differ in their site of localization in the host. While males usually occur in the serosa of the swimbladder, gravid and subgravid females of the same species are found, for example, in the host's abdominal cavity, fins or subcutaneous tissues. Therefore, it is interesting from this viewpoint that in *P. margolisi* the site of localization (gonads) is the same for both males and females.

Acknowledgements

We thank Prof. Clive Kennedy and Dr. Allister Lyndon from the University of Exeter, U.K., for reading the manuscript.

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Two new copepod species of the genus *Pseudotaeniacanthus* Yamaguti and Yamasu, 1959 (Poecilostomatoida: Taeniacanthidae), parasitic on moray eels in the Red Sea

S.C. Johnson and Z. Kabata

Abstract: Two new species of *Pseudotaeniacanthus*, ***P. margolisi*** n.sp. and ***P. septemsetigerus*** n.sp. (Copepoda, Poecilostomatoida, Taeniacanthidae), described and illustrated herein, are parasitic on the gills of unidentified species of moray eels (family Muraenidae) in the Gulf of Aqaba, Red Sea. *Pseudotaeniacanthus margolisi* differs from its closest congener in armature of the terminal segment of its fourth exopod and unique sexually dimorphic armature of its second leg. *Pseudotaeniacanthus septemsetigerus* is distinguished by the armature of its endopods, as well as by the proportions and armature of its caudal rami. A revised key to the species of the genus is appended.

Résumé : Deux nouvelles espèces de *Pseudotaeniacanthus*, ***P. margolisi*** n.sp. et ***P. septemsetigerus*** n.sp. (Copepoda : Poecilostomatoida : Taeniacanthidae), décrites et illustrées ici, parasitent les branchies d'espèces non identifiées de murènes (Muraenidae) dans le golfe d'Aqaba, en mer Rouge. Le *Pseudotaeniacanthus margolisi* diffère de son congénère le plus proche par l'armature du segment terminal de son quatrième exopodite et par le caractère particulier du dimorphisme sexuel de l'armature de sa deuxième patte. Le *Pseudotaeniacanthus septemsetigerus* se distingue par l'armature de ses endopodites ainsi que par les proportions et l'armature de ses rames caudales. On trouve en annexe une clé révisée des espèces du genre.

[Traduit par la Rédaction]

The genus *Pseudotaeniacanthus* Yamaguti and Yamasu, 1959, comprises five species of very small copepods, all parasitic on the gills of anguilliform fishes. The species are: *P. congeri* Yamaguti and Yamasu, 1959; *P. coniferus* Dojiri and Cressey, 1987; *P. longicauda* Pillai and Hameed, 1974; *P. muraenosocis* Uma Devi and Shyamasundari, 1980; and *P. puhi* Lewis, 1967. Lewis (1968) described also an unnamed male *Pseudotaeniacanthus*, which he found on *Acanthurus gahhm* (= *A. nigricans*), a perciform fish unrelated to anguilliforms. As a genus, *Pseudotaeniacanthus* is distinguished from its taeniacanthid confamilials by its unique rostral sclerites, Y-shaped, richly equipped with transverse rows of hooklets (or ridges), and in some species also with well-developed furca.

Judging from the fact that hitherto described species of *Pseudotaeniacanthus* are widespread among anguilliform fishes (members of four families: Congridae, Muraenidae, Muraenosocidae and Ophichthidae) and that their geographic range also appears extensive (Hawaii, Japan, India, and the Red Sea), it is easy to conjecture that more species of this genus are yet to be discovered. Their elusiveness is attributable to the relative inaccessibility of their hosts.

Examining the gills of unidentified moray eels (family Muraenidae), captured in the Gulf of Aqaba, Red Sea, in 1974, Dr. Ilan Paperna found some pseudotaeniacanthids, which he kindly sent us for examination. We were unable to place them in any of the previously known five species and are thus compelled to establish for them two new taxa, which herein are described and incorporated in a revised key to the genus.

Owing to the small size of these copepods, most of the handling and examinations had to be carried out under compound microscope. The study of the mouth parts required oil immersion lens. Berlese's fluid was used as a clearing agent and mounting medium. Drawings were made with the aid of a drawing tube. The excellent compendium of Dojiri and Cressey (1987) proved invaluable in our studies. We also consulted papers by Lewis (1967, 1968), Pillai and Hameed (1974), and Uma Devi and Shyamasundari (1980).

Descriptions

Pseudotaeniacanthus margolisi n.sp. Figs. 1–19

Record of specimens: Twenty five females and 11 males were taken by Dr. I. Paperna on 24 April 1974. A holotype female is deposited in the collections of the Smithsonian Institution (USNM cat. No 259705); an allotype male is

Received February 12, 1994. Accepted May 19, 1994
J12492

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deposited in the same collection (USNM cat. No. 259706), as are 15 paratype females and 10 paratype males (USNM cat. No. 259707).
Host: Unidentified moray eel (family Muraenidae).
Location: Gills.
Locality: Gulf of Aqaba, Red Sea.
Etymology: The specific name honors Dr. Leo Margolis of the Pacific Biological Station, Nanaimo, B.C.

Female (Fig. 1)
Cephalothorax wider than long, rounded anteriorly, with projecting, pointed, posterolateral corners and slightly concave posterior margin. Segmental boundaries between second and third, as well as third and fourth pedigerous segment not very distinct; second pedigerous segment about as long and wide as cephalothorax, third shorter, both with concave posterior margins; fourth narrower than preceding two, wider than long, suboval; fifth small, wider than long, with lateral papilliform processes forming bases of fifth legs; genital complex wider than long. Abdomen four-segmented, segments tapering caudad. Dimensions (in μm, based on nine specimens): total length 1122 (1030–1223); prosome length 714 (640–798), width 435 (394–492); genital complex length 90 (78–104), width 155 (129–172); abdomen length 408 (346–459), width 161 (123–196), caudal rami length 28 (24–31), width 28 (25–30).

First antenna (Fig. 2) six-segmented, boundaries between second and third segment indistinct; setal formula 6, 11, 7, 3, 2 + aesthete, 7 + aesthete. Second antenna (Fig. 3) four-segmented, with cubital joint between first and second segment; first segment with small seta at distal border, second with long, slender seta in distal half, third with row of spinules along outer margin, unciform spine and two pectinate processes at distal border, fourth with three setae and four claw-like spines at apex. Rostral area (Fig. 4) with Y-shaped sclerite, upper arms of Y bearing 11–12 transverse rows of spinules. Labrum membranous, without ornamentation. Mandible (Fig. 5) with two distal blades denticulated along one margin. Paragnath not observed. First maxilla (Fig. 6) small, lobate, with two long and three short setae, all unarmed. Second maxilla not observed. Maxilliped (Fig. 7) apparently three-segmented, basal segment (corpus) robust, bearing long unarmed seta, second segment fused with third, latter with two subequal, subapical setae.

Four pairs of biramous legs, all rami (except for two-segmented first exopod) three-segmented. Armature of rami as shown below:

	Exopod			Endopod		
	1	2	3	1	2	3
First leg	I–0	10	—	0–1	0–1	5
Second leg	I–0	I–1	III, 5	0–1	0–2	I, 4
Third leg	I–0	I–1	III, 5	0–1	0–2	I, 4
Fourth leg	I–0	I–1	III, 5	0–1	0–1	III

First leg (Fig. 8) with membranous fringe along lateral margin of sympod; segmentation of exopod indistinct. Second and third legs identical (Fig. 9), with short rows of needle-like spinules along distal margins of coxa and basis, fringes of spinules on lateral margins (except for basal segment of endopod, with fringe of fine setules), additional longitudinal rows of spinules on three segments of endopod; exopod spines with spinulated margins and whip-like tips, but distalmost spine semipinnate. Fourth leg differing from preceding two in having one seta less on second segment of endopod, as well as in armature of distal segment of that ramus (Fig. 10). Fifth leg (Fig. 11) two-segmented, uniramous, basal segment with short pinnate seta in distolateral corner, second segment with four naked setae at distal margin, row of fine spinules on distal half of medial margin and close to posterior margin. Sixth leg (Fig. 12) small, papilliform, with two longer and one very short naked setule. Caudal ramus (Fig. 13) subquadrate, bearing five setae, four on distal margin (second and third counting from medial side much longer than first and fourth), fifth seta short, distolateral.

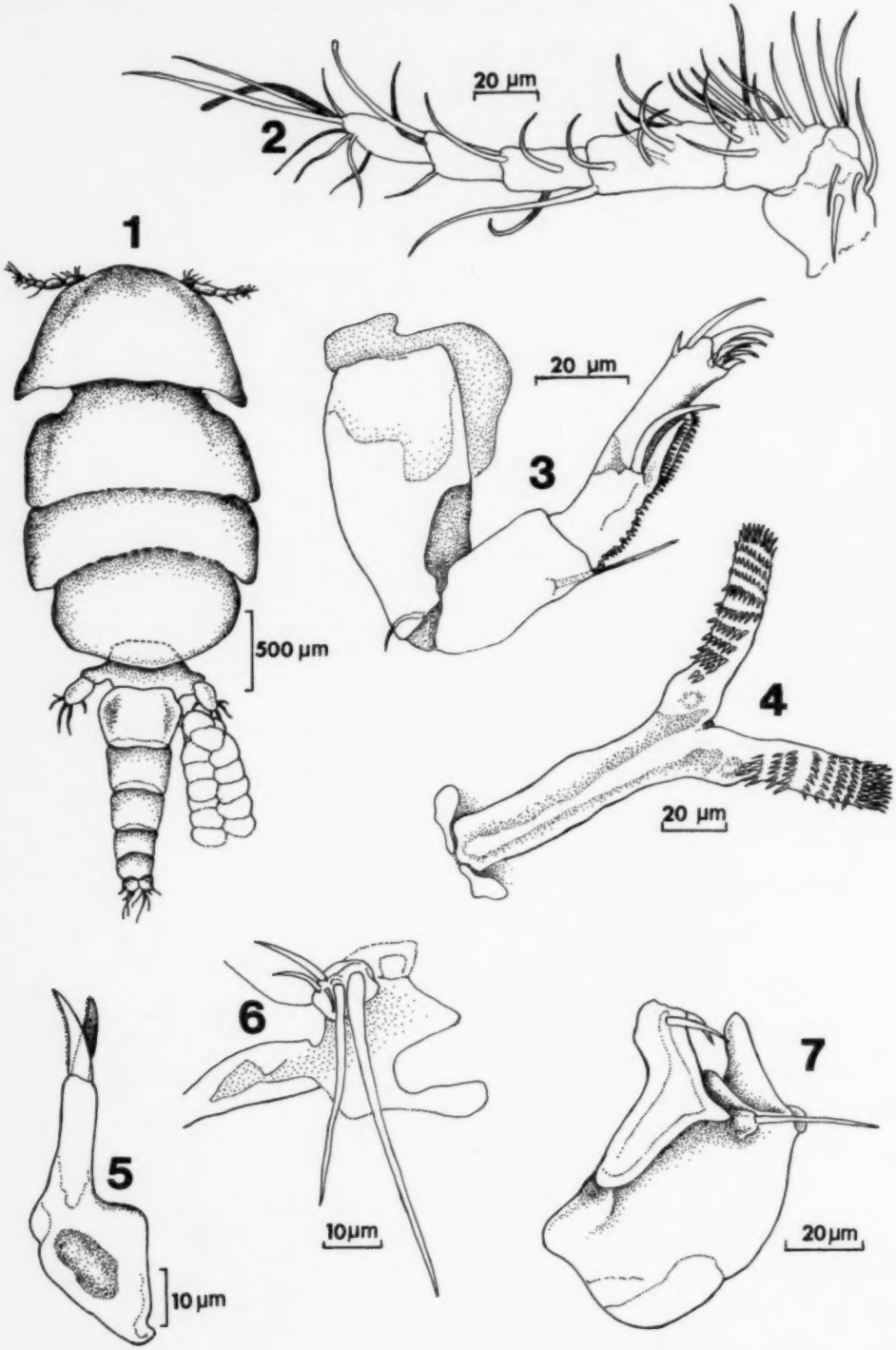
Male (Fig. 14)
Cephalothorax about equally long and wide, with rounded anterior and slightly concave posterior margin, with rounded posterolateral corners. Second pedigerous segment wider than cephalothorax, wider than long, with concave posterior margin; third narrower than second, fourth narrower than third, with convexly rounded posterior margin, fifth much smaller than fourth, wider than long. Genital complex subquadrate. Abdomen four-segmented, segments subquadrate, narrowing caudad. Dimensions (in μm, based on five specimens): total length: 1075 (991–1207); prosome length 666 (605–731), width 414 (372–448); genital complex length 100 (83–122), width 139 (131–146); abdomen length 410 (362–476), width 140 (101–150); caudal rami length 33 (30–36), width 28 (26–31).

Antennae and rostral sclerite as in female. Mandible (Fig. 15) differing from female appendage in having only one unarmed terminal blade and one subterminal, slender but stiff seta. Other buccal appendages as in female, but second maxilla not found. Maxilliped (Fig. 16) four-segmented, basal segment narrowing distally, with one long seta near mid-length; second segment (corpus) with powerful myxal outgrowth, concave at apex, bearing corrugated patch and two setae; third very small, unarmed; distal uncinate claw with truncated tip and two setae on proximal half.

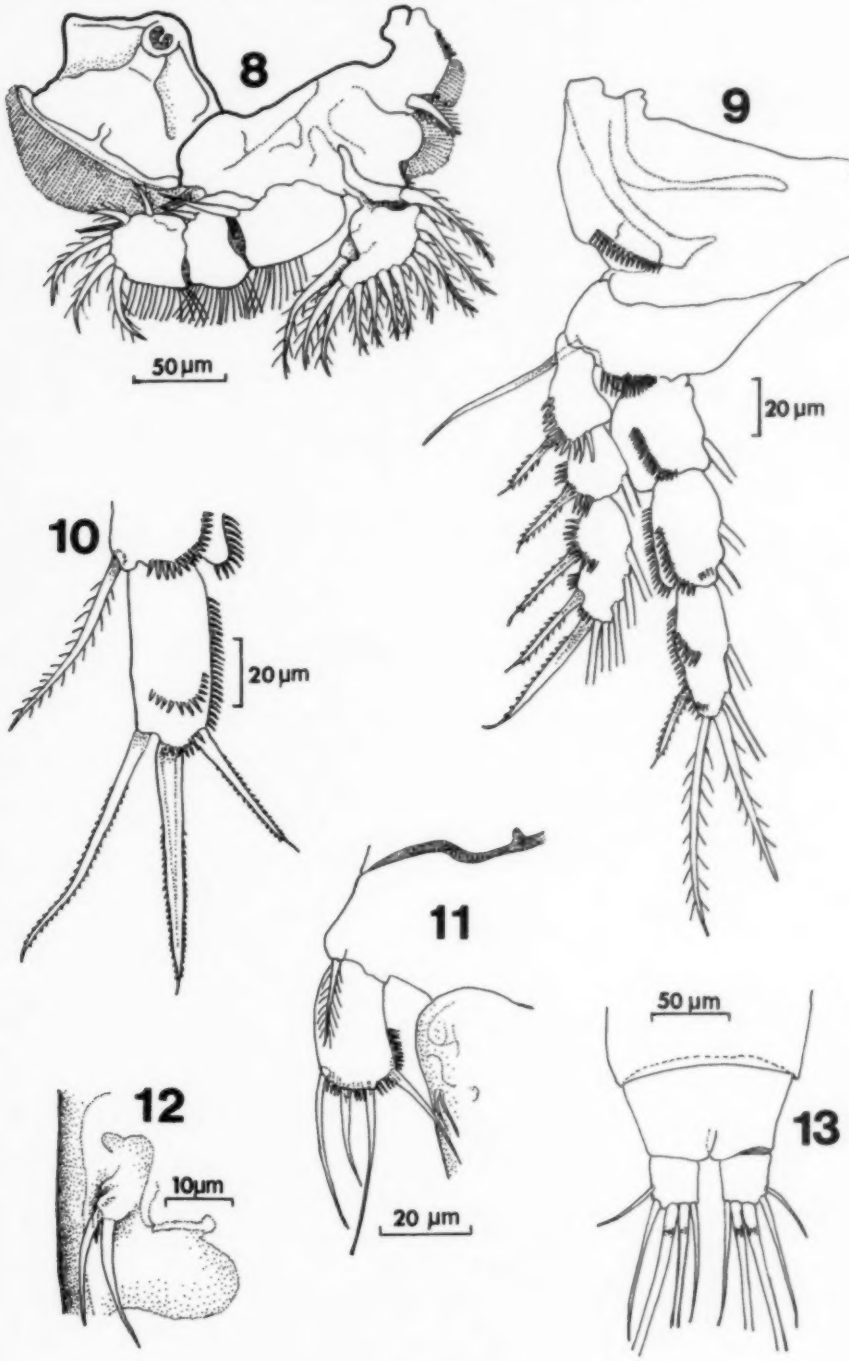
First four pairs of legs as in female, but with enlarged spines of second exopod (Fig. 17), spine of first segment with spinulated margins, remaining four spines unarmed, but with whip-like tips. Fifth leg (Fig. 18) with all setae naked and only short row of spinules in distolateral corner. Sixth leg (Fig. 19) represented by single seta. Caudal ramus as in female.

Comments
To establish specific identity of this species one must compare it with those of its congeners, with which it shares the following characteristics: (*i*) rostral sclerite with rows of spinules but without furca and (*ii*) subquadrate caudal ramus bearing five setae. Only one such species is known at present: *Pseudotaeniacanthus puhi* Lewis, 1967. The first

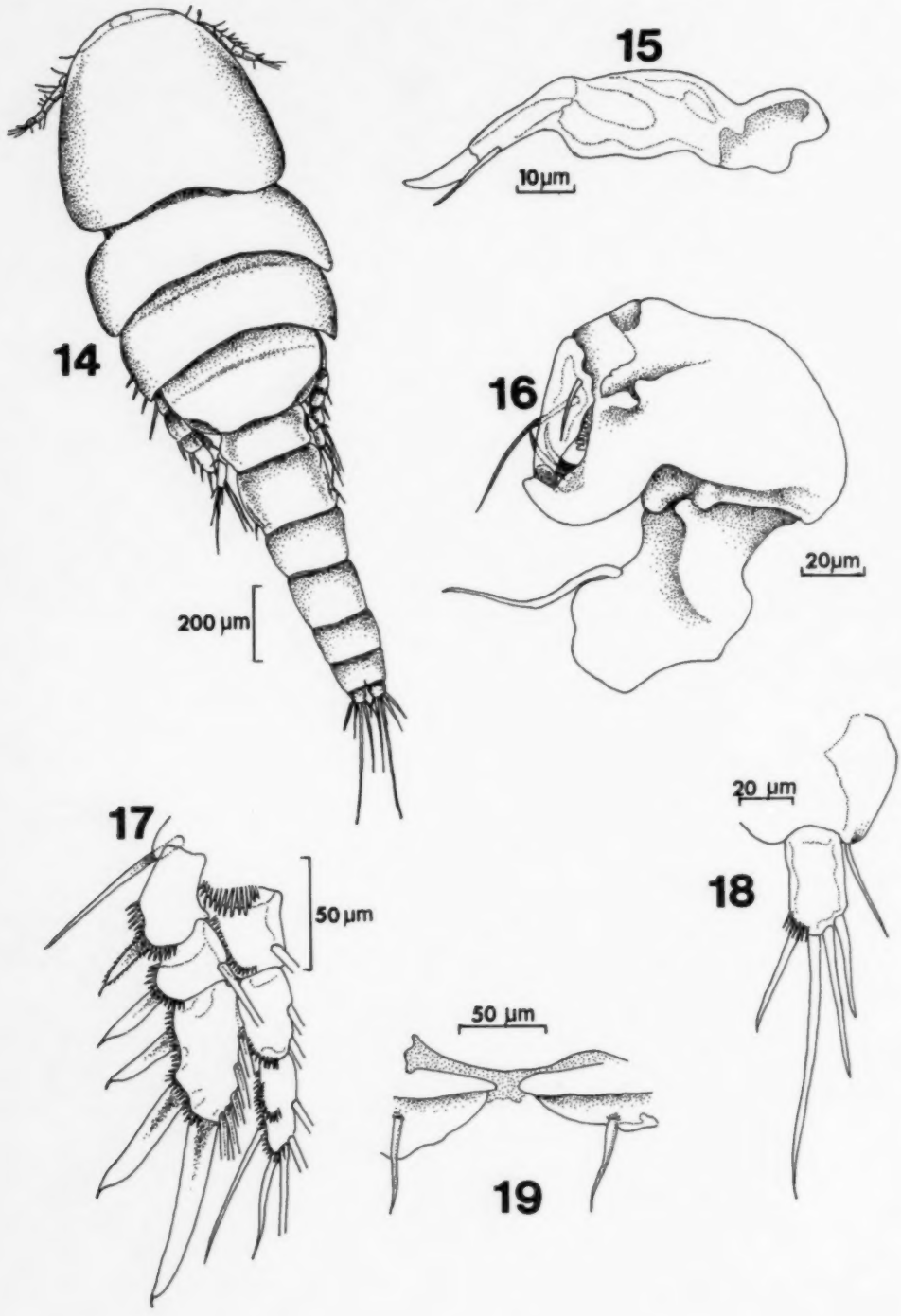
Figs. 1–7. *Pseudotaeniacanthus margolisi* n.sp. Fig. 1. Female, entire, dorsal. Fig. 2. First antenna, ventral. Fig. 3. Second antenna, ventral. Fig. 4. Rostral sclerite, ventral. Fig. 5. Mandible, ventral. Fig. 6. First maxilla, ventral. Fig. 7. Maxilliped, ventral.



Figs. 8–13. *Pseudotaeniacanthus margolisi* n.sp. Fig. 8. First leg, anterior. Fig. 9. Second leg, anterior. Fig. 10. Fourth leg, distal half of endopod, anterior. Fig. 11. Fifth leg, ventral. Fig. 12. Sixth leg, ventral. Fig. 13. Caudal rami and extremity of abdomen, ventral.



Figs. 14–19. *Pseudotaeniacanthus margolisi* n.sp. Fig. 14. Male, entire, dorsal. Fig. 15. Mandible, ventral. Fig. 16. Maxilliped, ventral. Fig. 17. Rami of second leg, anterior. Fig. 18. Fifth leg, ventral. Fig. 19. Sixth leg, ventral.



obvious difference between these two species is in their habitus; *P. puhi* is more slender than the specimens described above. The rostral sclerite of *P. puhi* bears 9–10 transverse rows of spinules on its diverging arms, that of *P. margolisi* bears 11–12. The two species differ also in some details of the armature of the second to fourth legs. Terminal setae of the fifth leg of *P. puhi* are pinnate, those of *P. margolisi* are unarmed. Finally, the armature of the second leg of *P. margolisi* shows sexual dimorphism unique for the genus. Enlarged spines of the exopod of this leg occur in the male, but not in the female of this species. In *P. puhi* they are enlarged in both sexes.

In view of these differences, we deem it justifiable to accord to *P. margolisi* the status of an independent taxon.

It should also be noted that in spite of assiduous search in several specimens we were unable to locate the second maxilla of this species, although this appendage is usually easier to see than the first maxilla, which we did find without difficulty. While our inability to find it might be attributable to our inadequate powers of observation, it is at least possible that *P. margolisi* is devoid of the second maxilla. Its absence would make this species unique in its genus. The fact that the absence of the second maxilla could not be established beyond doubt prompted us to exclude this apparent characteristic from the key to the species of the genus.

Pseudotaeniacanthus septemsetigerus n.sp. Figs. 20–34
Record of specimens: Thirteen females were taken by Dr. I. Paperna on 24 April 1974. A holotype female is deposited in the collections of the Smithsonian Institution (USNM cat. No. 259708); eight paratype females (USNM cat. No. 259709) are deposited in the same collection.
Host: Unidentified moray eel (family Muraenidae)
Location: Gills.
Locality: Gulf of Aqaba, Red Sea.
Etymology: The specific name combines two Latin words, *septem* = seven, and *setigerus* = bearing setae. It alludes to the presence of seven setae on the caudal rami of this species.

Female (Fig. 20)
Cephalothorax wider than long, rounded at anterior end, with bluntly subtriangular posterolateral corners (in Fig. 20 damage in the left anterolateral corner area indicated by an arrow). Second pedigerous segment about as wide as cephalothorax, its length less than half its width; third segment narrower but equally short; fourth suboval, narrower than third; fifth small, wider than long; genital complex subquadrate. Abdomen four-segmented, segments subquadrate, narrowing caudad. Dimensions (in μm, based on five specimens): total length 1441 (1323–1516); prosome length 929 (849–982), width 605 (577–620); genital complex length 125 (108–144), width 208 (186–236); abdomen length 541 (474–608), width 245 (221–276); caudal rami length 51 (49–57), width 34 (32–37).

First antenna (Fig. 21) six-segmented, indistinct boundaries between first three segments, with geniculate flexion between first and second segments; setal formula 6, 10, 7, 3, 2 + aesthete, 7 + aesthete. Second antenna (Fig. 22) apparently three-segmented (terminal segment probably representing fused third and fourth), with cubital joint between first and second segment, each bearing single seta; terminal segment with denticulated inner margin, two pectinate processes denticulated on one margin and unciform claw at midlength, four claw-like spines and two setae at apex. Rostral sclerite (Fig. 23) without furca, bearing seven rows of hooklets on each anterior divergent branch. Labrum membranous, its rounded posterior margin bearing row of spinules, labial area unarmed. Mandible (Fig. 24) with distal half flexed caudad and two terminal blades denticulated along posterior margins. Paragnath (Fig. 25) roughly semicircular, with crescentic row of denticles close to apex. First maxilla (Fig. 26) small lobe, with two long setae bearing minute denticles, and two short setules. Second maxilla (Fig. 27) two-segmented, basal segment robust, second slender, tipped with acuminate blade and short, unarmed seta. Maxilliped (Fig. 28) three-segmented, basal segment bearing long naked seta, second unarmed, third very small, conical, with two long subequal setae, one apical and one subapical.

Four pairs of biramous legs, all rami (except for two-segmented first exopod) three-segmented. Armature of rami as shown below:

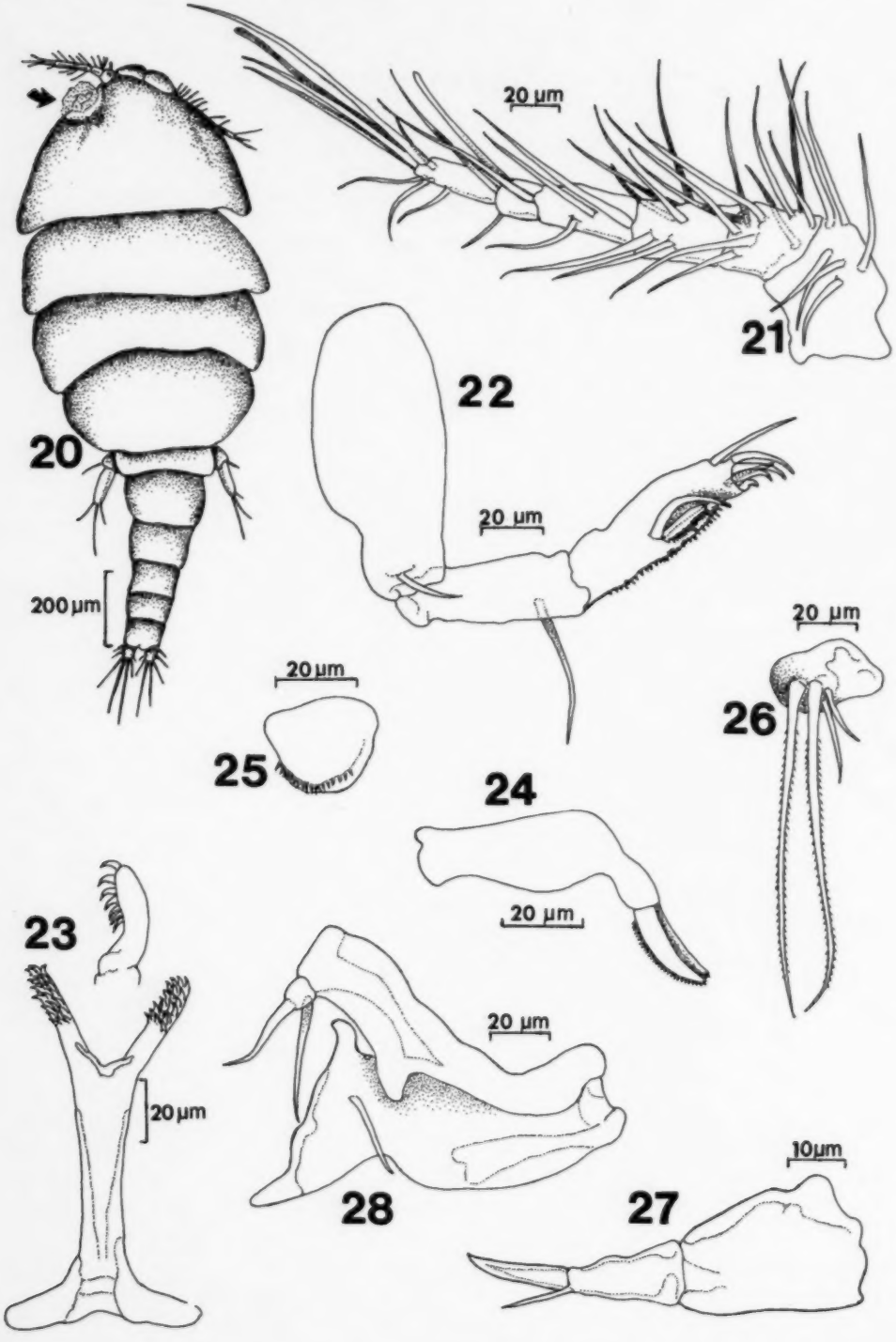
	Exopod			Endopod		
	1	2	3	1	2	3
First leg	I–0	10	—	0–1	0–1	5
Second leg	I–0	I–1	III, 5	0–1	0–2	I, 4
Third leg	I–0	I–1	III, 5	0–1	0–2	I, 4
Fourth leg	I–0	I–1	III, 5	0–1	0–1	III

Segmentation of first exopod indistinct (Fig. 29). First leg with fringe of spiniform setules along lateral margin, except in its distal part with membrane and fine setules. Second and third legs identical (Fig. 30), with fringes of spiniform setules on lateral margins of all exopod and distal endopod segments; first two endopod segments with fringes of fine setules; rows of spiniform setules also on ventral surfaces of distal exopod segment and all three endopod segments; exopod spines with rows of fine spinules on margins. Fourth leg (Fig. 31) differing from preceding two in having one seta less on second segment of endopod, as well as in armature of its distal segment. Fifth leg (Fig. 32) two-segmented, uniramous, first segment with pinnate seta in distolateral corner, second with four such setae on distal margin and two short rows of spinules in distolateral corner and close to distal margin. Sixth leg (Fig. 33) represented by three short naked setae. Caudal rami (Fig. 34) bearing seven setae: two on lateral margin, one close to medial margin, four on distal margin (two central ones much longer than other two).

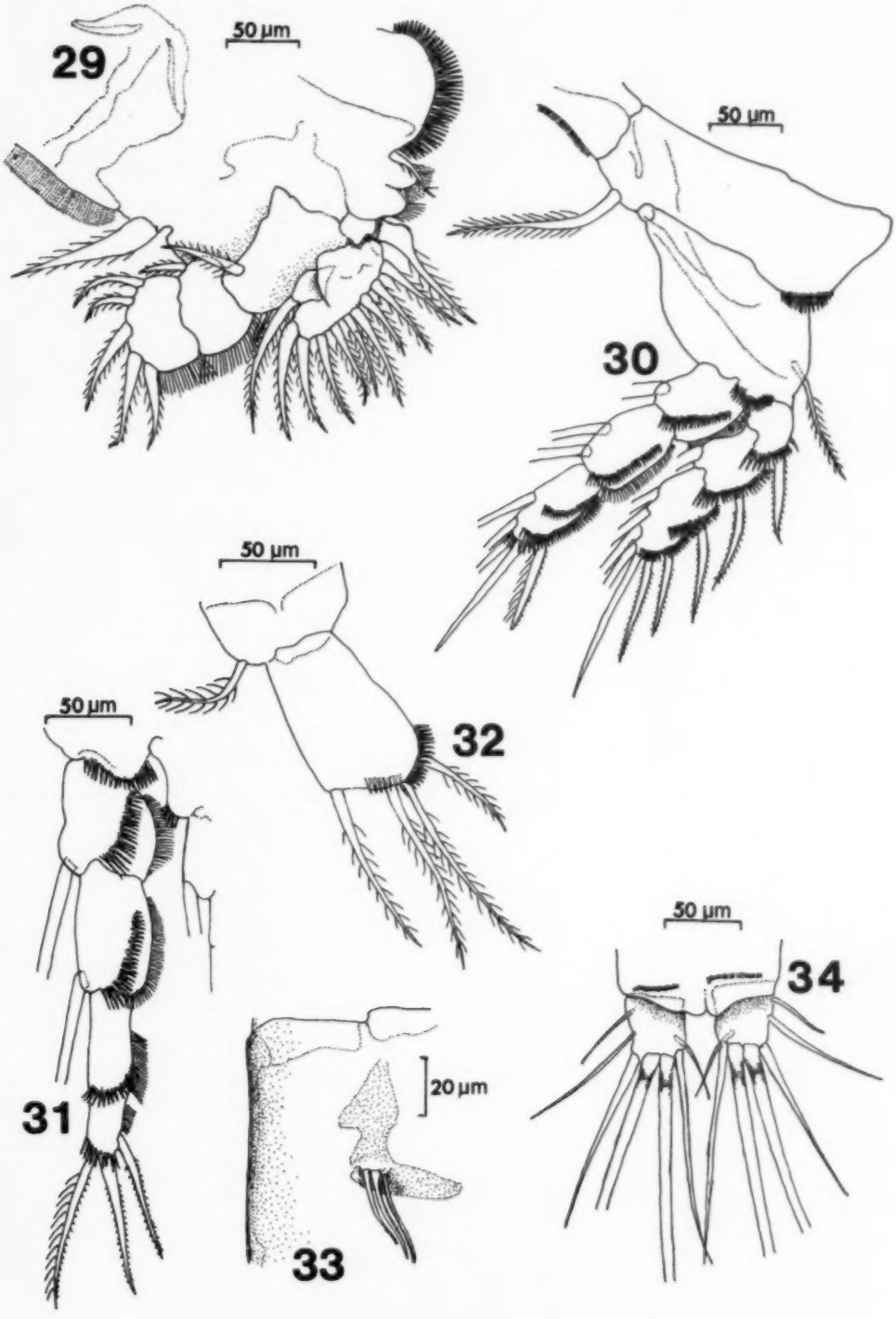
Male: Unknown

Comments
The presence of seven setae on the caudal rami of this species (Fig. 34) distinguishes it from all other species of *Pseudotaeniacanthus*, except *P. longicauda*. That species,

Figs. 20–28. *Pseudotaeniacanthus septemsetigerus* n.sp. Fig. 20. Female, dorsal (damaged area indicated by arrow). Fig. 21. First antenna, ventral. Fig. 22. Second antenna, ventral. Fig. 23. Rostral sclerite, ventral. Fig. 24. Mandible, ventral. Fig. 25. Paragnath, ventral. Fig. 26. First maxilla, ventral. Fig. 27. Second maxilla, ventral. Fig. 28. Maxilliped, ventral.



Figs. 29–34. *Pseudotaeniacanthus septemsetigerus* n.sp. Fig. 29. First leg, anterior. Fig. 30. Second leg, anterior. Fig. 31. Fourth leg, endopod, anterior. Fig. 32. Fifth leg, ventral. Fig. 33. Sixth leg, ventral. Fig. 34. Caudal rami, ventral.



however, has caudal rami almost as long as the terminal segment of the abdomen, whereas the length of these structures in *P. septemsetigerus* does not exceed twice their own width; i.e., they are much shorter than the terminal segment of the abdomen. The two species differ from each other also in the setation of the second, third and fourth endopods. These characteristic differences, as well as differences in general habitus, suffice to postulate for *P. septemsetigerus* the status of an independent taxon.

Key to the species of *Pseudotaeniacanthus*

1. Caudal rami bearing five setae 2
   Caudal rami bearing more than five setae 4
2. Rostral sclerite with rows of hooklets and furca ..... *P. muraenosocis*
   Rostral sclerite with rows of hooklets, furca absent 3
3. Terminal segment of fourth exopod with three spines; exopod spines enlarged in males only .. *P. margolisi*
   Terminal segment of fourth exopod with one spine and two pinnate setae; exopod spines enlarged in both sexes ..... *P. puhi*
4. Caudal rami bearing six setae 5
   Caudal rami bearing seven setae 6
5. Prosome shorter than urosome; length of caudal ramus more than five times greater than its width ..... *P. coniferus*
   Prosome slightly longer than urosome; length of caudal ramus not exceeding twice its width ... *P. congeri*
6. Prosome shorter than urosome, length of caudal ramus more than four times greater than its width ..... *P. longicauda*
   Prosome longer than urosome, length and width of caudal ramus subequal ..... *P. septemsetigerus*

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The poor potential of cunner, *Tautogolabrus adspersus*, to act as cleaner fish in removing sea lice (*Caligus elongatus*) from farmed salmon in eastern Canada

B.M. MacKinnon

Abstract: Cunner, *Tautogolabrus adspersus*, were found not to be good candidates for removal of sea lice, *Caligus elongatus*, from farmed Atlantic salmon (*Salmo salar*) during seapen trials conducted in Passamoquoddy Bay, N.B. In laboratory trials conducted in 30-gal (~136 L) glass tanks, using one cunner per infected salmon, a significant reduction in sea lice numbers was evident. In seapen trials using 30 cunner to 2000 Atlantic salmon, no significant reduction in sea lice numbers was evident after 12 weeks. Availability of fouling organisms on the seapen net may account, in part, for the lack of cleaning behaviour in these fish.

Résumé : La tanche-tautogue, *Tautogolabrus adspersus*, na pas donné de bons résultats pour l'élimination des poux de poisson, *Caligus elongatus*, présents sur des saumons atlantiques (*Salmo salar*) d'élevage lors d'essais réalisés en enclos dans la baie Passamaquoddy (N.-B.). Dans des essais de laboratoire effectués dans des aquariums de 30 gal (env. 136 L), avec une tanche-tautogue par saumon infecté, on avait observé une réduction notable du nombre de poux de poisson. Dans les enclos contenant 30 tanches-tautogues pour 2000 saumons atlantiques, on n'a observé aucune réduction notable du nombre de poux de poisson après 12 semaines. Il se peut que la disponibilité des salissures présentes sur le filet de l'enclos explique en partie l'inefficacité de ces poissons.

[Traduit par la Rédaction]

Introduction

Sea lice are ectoparasitic copepods that can cause severe pathology and mortality in salmon. Aquaculture increases the likelihood of heavy infections with sea lice due, in part, to the abundance of potential hosts and to increased stress of the fish. *Lepeophtheirus salmonis* Kroyer, 1838, common in Britain, Norway, and western Canada, tends to be more pathogenic than *Caligus elongatus* von Nordmann, 1832, a smaller species that is common in eastern Canada. While Aquagard®, an organophosphate, is licensed for use in Europe, no drugs have as yet been approved for use in Canada. In an attempt to find treatment alternatives to drugs, salmon farmers in Scotland, Ireland, and Norway have experimented with and are having success using cleaner fish to remove sea lice from farmed salmonids (Bjordal 1990, 1992; Costello and Bjordal 1990; Costello and Donnelly 1990; Treasurer 1991, 1993).

The only likely candidate for cleaning behaviour in eastern Canada is the cunner, *Tautogolabrus adspersus*. Cunner are in the family Labridae (the wrasses) and inhabit shallow inshore waters of the northwest Atlantic Ocean. They mature at 8–11 cm in length, grow to a maximum of 43 cm, and may live up to 10 years. They feed mainly on molluscs and crustaceans, occasionally on sea urchins, marine worms, fish eggs, and eelgrass (Scott and Scott 1988). Since *Tautogolabrus* is in the same family as *Ctenolabrus* and *Centrolabrus*, the two genera containing species currently being used as cleaner fish on salmon farms in Scotland, Ireland, and Norway (Treasurer 1993), the aim of this study was to assess the ability of *T. adspersus* to act as cleaner fish to remove sea lice from farmed Atlantic salmon (*Salmo salar*) in New Brunswick.

Received March 7, 1994. Accepted December 6, 1994
J12673

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Materials and methods

Forty cunner were caught by hook and line in the St. Croix estuary in New Brunswick in mid-June 1993. They were kept alive in aerated sea water for approximately 4 h prior to transfer to a 1.5 m diameter holding tank with running sea water. The smallest and the largest fish were anaesthetized with MS222 and their lengths recorded. The remainder of the fish were not similarly handled, since the effects of such handling were unknown for this species and it was important to keep as many of these fish alive as possible. They were fed ad libitum with crushed fresh

clams and occasionally small periwinkles and were provided with 7–10 cm diameter plastic drain tubing for "hides." Cunner were used in laboratory experiments within the first 8 weeks of capture.

Laboratory experiments were conducted using 30-gal (~136 L) glass tanks with running natural sea water at ambient light and temperature (8–11°C over the course of the experiment). Each tank contained a 10 cm diameter plastic tube which the cunner used as a "hide." Cunners were selected at random from the holding tank, one cunner being placed in each experimental tank, deprived of food, and left to acclimatize for 24 h.

Small Atlantic salmon smolts, 18–22 cm in length, were infected with adult *C. elongatus* that had been collected from naturally infected salmon no more than 4 h earlier, by placing each salmon in a plastic tank containing 5 L of aerated, ambient temperature sea water and approximately 50 sea lice. Salmon were removed from the infection tank after 15 min. To avoid handling infected fish and their attached sea lice, the numbers of sea lice on each salmon were not equalized, and salmon were gently netted into test and control tanks.

One infected salmon was added to each tank containing a cunner and two salmon were placed in control tanks without cunner. This was done to equalize the test and control situations so that two fish would be in each tank. The number of sea lice on each infected salmon was counted after salmon were placed in the tanks. Salmon in test and control tanks were checked after 24 h and the number of sea lice on each salmon recorded. Twenty cunner-salmon trials were completed and 10 salmon-salmon trials were used. Individual salmon in the control tanks were identified by noting natural differences in body markings. After each experiment the cunner and salmon were placed in new holding tanks so that they would not be used again.

Seapen trials were conducted in early September. Thirty cunner were transferred to seapen cages at the Atlantic Salmon Development and Demonstration Farm in Limekiln Bay, N.B. All were put into a 12 m square pen with 2000 smolts. A control seapen beside the test pen also contained 2000 smolts. The salmon in both of these seapens had been regularly surveyed for sea lice throughout the summer and were known to carry sea lice, the average numbers of which were not statistically different (35 ± 16 for test seapen, 41 ± 12 for control seapen in September). Twelve weeks later salmon from both pens were monitored for intensity of infection by sea lice. The cage nets were drawn up to allow easy catching of the salmon. One hundred salmon from each seapen were individually dip-netted and hand-held over a tub filled with sea water (in case the fish wiggled free). Sea lice were counted from the entire surface of each fish.

For statistical analysis of data obtained from laboratory trials, the change in the number of sea lice on each fish after 24 h was calculated. The change in sea lice numbers in the paired salmon controls was averaged for each pair. The Mann–Whitney test for nonparametric data was used to compare the change in numbers of sea lice on salmon held with cunner to the change in numbers of sea lice on salmon held with another salmon. Student's *t*-test was used to compare the numbers of sea lice on the 100 fish taken from each of the seapens.

Results

The change in the number of sea lice on each salmon after 24 h was significantly greater ($P < 0.05$) for salmon held with cunner ($\bar{X} = 2.4$) than for salmon held with another salmon ($\bar{X} = 0.4$) (SE = 0.7). This suggests that under laboratory conditions cunner significantly reduced the number of sea lice on salmon held in the same tank.

The numbers of sea lice on salmon from seapens either with ($\bar{X} = 42.00$) or without cunner ($\bar{X} = 43.05$) were not significantly different ($t_{calc.} = 0.75$; $p = 0.2$; SE = 2.85). This suggests that, under these experimental conditions, *T. adspersus* was not effective in reducing sea lice infections on salmon held in seapens.

Discussion

Although laboratory trials showed a significant difference in sea lice numbers on salmon held with cunner compared with those held with other salmon, the results of individual trials were dichotomous, in that all, or most, sea lice were removed from salmon by some cunner and no lice were removed by others. Although there is no evidence that cunner show cleaning behaviour in the wild, differences in the behaviour of individual fish in the laboratory may have resulted from capture and handling, which could have affected some fish more than others. Cleaning behaviour under laboratory conditions may be a feature determined by individual experiences and preferences rather than a "typical" species-wide feeding behaviour. While discussions in Europe regarding the potential value of establishing cleaner fish hatcheries are still at the conjecture stage, a breeding program of cunner might, theoretically, be able to select cleaning individuals for propagation.

The failure of cunner to clean in the seapen trials may merely indicate that cleaning other fish is not their normal feeding behaviour and that they prefer to eat small molluscs and crustaceans when these are available. The presence of fouling organisms on the seapen nets may have reduced the need for cunners to seek alternative food resources, such as sea lice.

The stocking density used in this study was comparable to the lower stocking densities used in wrasse studies in Norway. Bjordal (1990), for example, used densities of one wrasse to 43 salmon. It may be that since only a proportion of the cunner in the present experiment showed cleaning behaviour, the present stocking densities may have been too low to make a difference in sea lice numbers in the seapen. The average numbers of sea lice in both seapens were so similar, however, as to suggest that none of the cunner were eating sea lice from the salmon.

Cunner less than 10 cm in length have a dark spot in front of the soft dorsal fin (Scott and Scott 1988), and spots of this nature are considered to be common on cleaner fish. Thus it may be that younger cunner show cleaning behaviour, while older fish may be less inclined to this mode of feeding. Most cunner used in this experiment were larger than 10 cm and did not have a black mark.

If younger cunner were used, at higher stocking densities, and if the nets on the seapens were kept completely clean, it may be that this species could remove sea lice from salmon. As a commercial prospect, however, the use of cunner is not recommended at this time since (*i*) large numbers of cunner are difficult to collect; (*ii*) small cunner, potentially able to clean, would probably lose their cleaning behaviour as they grow larger over the summer months and would be more likely to escape through the seapen nets or to be eaten by the larger salmon; and (*iii*) unless specially treated nets are used that prevent fouling for several months, organisms would settle very quickly on seapen nets at all farms sites, thus providing the preferred food items for cunner.

Acknowledgements

Mr. Fred Purton and Dr. Michael Burt kindly caught the cunner used in these experiments. The assistance of the staff at the Huntsman Marine Science Centre, St. Andrews, N.B., and the New Brunswick Salmon Development and Demonstration Farm, St. George, N.B., was greatly appreciated. Dr. Gerry Friars, of the Atlantic Salmon Federation, kindly allowed us to use his fish for these trials. This research was funded by the New Brunswick Salmon Growers Association and by an AFAP grant from the Department of Fisheries and Oceans, Canada.

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Rediscovery in Japan and host association of *Salmincola carpionis* (Copepoda: Lernaeopodidae), a parasite of wild and reared freshwater salmonids

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Abstract: The parasitic copepod *Salmincola carpionis* (Krøyer, 1837) was rediscovered in Japan, where this parasite occurred on species of the genus *Salvelinus* (whitespotted char, *S. leucomaenis*; brook trout, *S. fontinalis*; their natural hybrid; Dolly Varden, *S. malma*; Miyabe char, *S. malma miyabei*) and rarely on rainbow trout (*Oncorhynchus mykiss*). The copepods were found on salmonids from a river in central Honshu and on those reared at an aquarium and a fisheries experimental station in northern Honshu. The preferred attachment site was the buccal cavity but at high intensities copepods were a little more frequently found on other parts. Prevalence and mean intensity of infection increased with increased size and age of the fish. At a constant water temperature, there were no seasonal changes in prevalence or mean intensity of infection, which may have resulted from continuous egg production by the copepods. The copepods that were previously described as *Salmincola falculata* (Wilson, 1908) and later identified as *S. californiensis* (Dana, 1852) from kokanee (*O. nerka*) from Hokkaido, Japan, are assigned to *S. carpionis*.

Résumé : Le copépode parasite *Salmincola carpionis* (Krøyer, 1837) a été redécouvert au Japon sur *Salvelinus* spp. [*S. leucomaenis*; *S. fontinalis* (omble de fontaine); leur hybride naturel; *S. malma* (Dolly Varden); *S. malma miyabei*) et, plus rarement, sur *Oncorhynchus mykiss* (truite arc-en-ciel). Les copépodes ont été trouvés sur des salmonidés provenant d'une rivière du centre de Honshu et sur des salmonidés élevés dans un aquarium et dans une station de pêche expérimentale du nord de Honshu. Le siège d'attache de prédilection était la cavité buccale mais chez les poissons les plus gravement infectés, les copépodes étaient présents en nombres légèrement plus élevés sur d'autres parties du corps. La prévalence et l'intensité moyenne de l'infection augmentaient en fonction de la taille et de l'âge des poissons. Chez les poissons exposés à une température de l'eau constante, aucun changement saisonnier de la prévalence ou de l'intensité de l'infection imputable à une production ininterrompue d'oeufs par les copépodes n'a été observé. Les copépodes décrits antérieurement comme *Salmincola falculata* (Wilson, 1908) et identifiés ultérieurement comme *S. californiensis* (Dana, 1852) à partir de kokanis (*O. nerka*) provenant de Hokkaido, au Japon, sont considérés ici comme *S. carpionis*.
[Traduit par la Rédaction]

Copepods of the lernaeopodid genus *Salmincola* are mainly parasites of freshwater salmonids and related fishes in the northern hemisphere (Kabata 1969). Seventeen species are currently known in this genus (Kabata 1988). To date, three named and one unidentified species have been recorded from a variety of salmonids from Japan (Nagasawa et al. 1987, 1994; Nagasawa and Urawa 1991): *Salmincola californiensis* (Dana, 1852) (syn. *S. falculata* (Wilson, 1908) and *S. yamame* Hoshina and Suenaga, 1954) from kokanee (landlocked form of sockeye salmon, *Oncorhynchus*

Received February 1, 1994. Accepted December 12, 1994.
J12665

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nerka), cherry salmon (river resident or landlocked form, *O. masou*), and whitespotted char (*Salvelinus leucomaenis*, formerly *S. leucomaenis pluvius*); *Salmincola carpionis* (Krøyer, 1837) from Dolly Varden (*Salvelinus malma*); *Salmincola stellatus* Markevich, 1936 from Japanese huchen (*Hucho perryi*); and *Salmincola* sp. from whitespotted char and rainbow trout (*O. mykiss*, syn. *Salmo gairdneri*). Of these species, the occurrence of *S. carpionis* in Japan has been regarded as questionable (Nagasawa et al. 1987), because, although Markevich (1956) reported one of the localities for this species as "Japan", in an earlier publication (Markevich 1940) he simply reported it as occurring in "the Far East," viz., there is a possible discrepancy between the two localities given by Markevich (1940, 1956), and there was no detailed locality reported in 1956. Since his record, *S. carpionis* has never been reported from Japan.

During a study on the taxonomy and ecology of *Salmincola* spp. parasitic on freshwater salmonids in Japan, we obtained specimens that are assigned to *S. carpionis*. Herein, we redescribe *S. carpionis* based on this newly collected Japanese material. We also present information on its occurrence on various salmonids and other freshwater fishes under wild and artificial conditions, in relation to season and host age or size.

Materials and methods

Fishes were obtained at three sites in central and northern Honshu, Japan: the Azusa River, Kamikochi, Nagano Prefecture (36°15′N, 137°40′E); the Iwate Prefectural Inland Fisheries Experimental Station, Matsuo, Iwate Prefecture (39°57′N, 141°03′E) (hereafter referred to as the Matsuo Laboratory); and the Asamushi Aquarium, Asamushi, Aomori Prefecture (40°53′N, 140°52′E). To determine the host preference of *S. carpionis*, various salmonids and other fishes were examined at the Azusa River from August 1989 to October 1991, at the Asamushi Aquarium in October 1986, and at the Matsuo Laboratory from March 1984 to May 1985. Fishes were measured (fork or body length) and weighed, and the number of *S. carpionis* counted and their sites of attachment recorded.

Monthly collections were made at the Matsuo Laboratory from June 1984 to May 1985 to study the seasonal occurrence of *S. carpionis* on three species of salmonids, viz., *S. leucomaenis*, *O. mykiss*, and *O. masou*. Fishes were anesthetized in tricaine methanesulfonate (MS 222) and processed as described above.

For identification and measurements, copepods were fixed and preserved in 70% ethanol or 5% formalin. Specimens from whitespotted char of the Asamushi Aquarium were cleared and dissected in 85% lactic acid by the wooden slide procedure of Humes and Gooding (1964). Measurements were taken from 10 Asamushi specimens from whitespotted char that were soaked in lactic acid and are given in millimetres with means in parentheses. For the trunk length, an additional 40 Asamushi Aquarium specimens from whitespotted char were also measured. All drawings were made with the aid of a camera lucida. Voucher specimens from the Asamushi Aquarium are deposited in the National Science Museum, Tokyo (NSMT-Cr 11284).

Terminology used for the morphology is that adopted by Kabata (1979). Two parameters, prevalence (percentage of hosts infected) and mean intensity (mean number of parasites per infected host), defined by Margolis et al. (1982), are used to describe the levels of parasitic infection. The common names of fishes follow those preferred by the American Fisheries Society.

Prevalence data were compared using *G*-tests of independence. When the *G*-values were marginally significant, they were adjusted with Williams' correlation (Sokal and Rohlf 1981). A one-way analysis of variance (ANOVA) was used to determine the effect of fish size or age on intensity of infection. Differences were considered significant at $p < 0.05$.

Results

Description of female (Fig. 1)

Cephalothorax (Fig. 2) ovoid, wider posteriorly, 1.35–1.93 (1.64) at widest portion. Trunk roughly oval, 3.00–4.70 (3.83) × 2.16–2.86 (2.51), with distinct, narrow neck-like anterior part, 0.60–1.00 (0.79) long, and rounded margins. A minute genital process at posterior end. Egg sacs mostly slender, 3.55–7.50 (5.58) × 0.78–1.13 (0.95), sometimes slightly ovoid, 2.45–2.66 (2.56) × 0.87–1.13 (1.00). Eggs 0.26–0.34 (0.30) in diameter. Total length (excluding egg sacs) 4.15–6.45 (5.02).

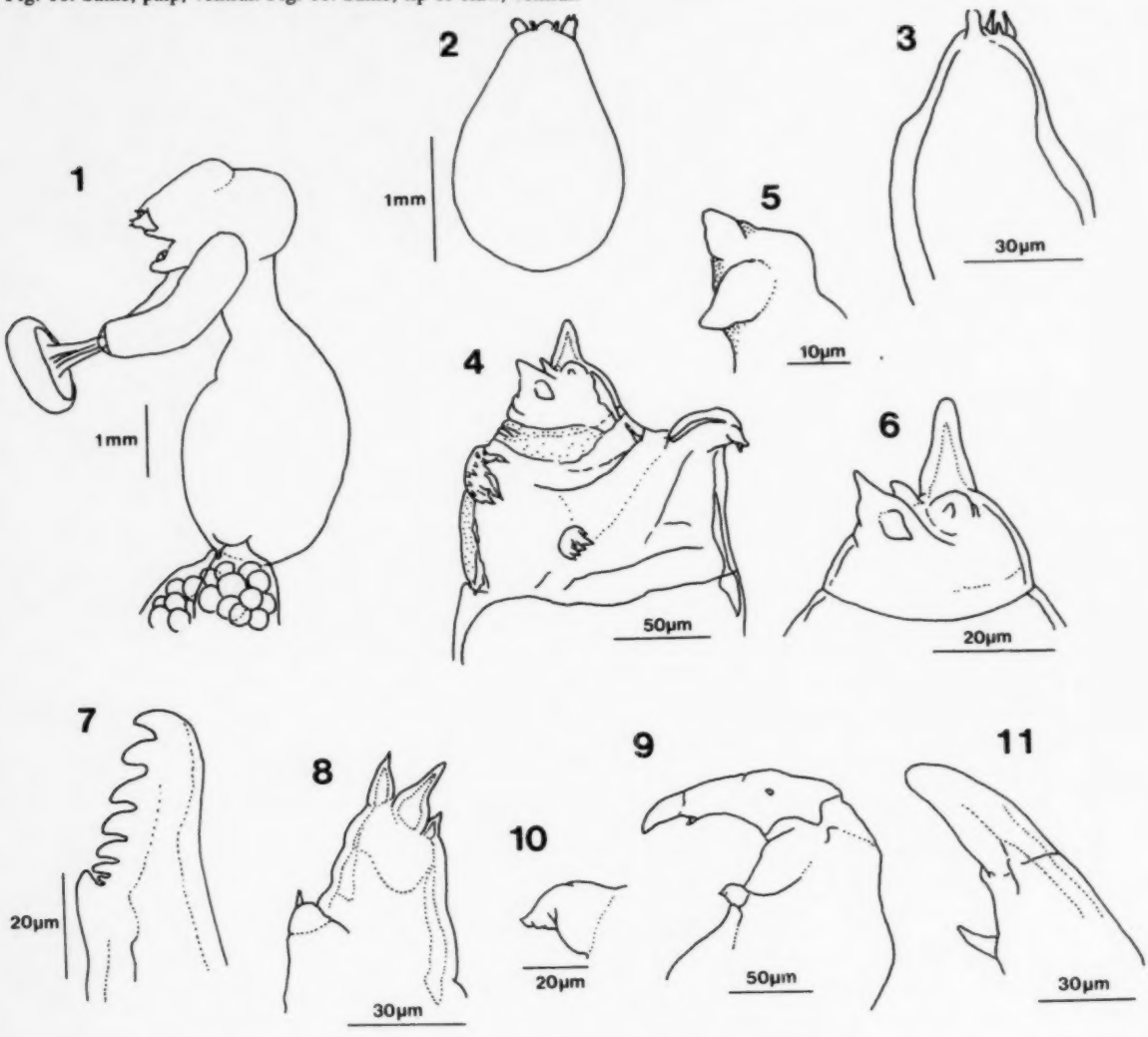
First antenna (Fig. 3) with poorly developed apical armature (four processes recognized in specimens examined) short, plump; and obscurely two segmented with inflated basal part. Second antenna (Fig. 4) with spines on spiny pad of sympod and of basal segment of endopod; exopod bearing two papillae near its tip (Fig. 5); endopod with apical armature (Fig. 6) comprised of hook, medial seta, lateral papilla, and two ventral processes. Mandible (Fig. 7) with seven teeth. First maxilla (Fig. 8) with lateral, prominent exopod with small setule on its apex; endopod with three short terminal papillae. Second maxillae subcylindrical, 2.28–4.25 (3.02) long. Maxilliped (Fig. 9) with small palp of irregular shape (Fig. 10); claw of subchela (Fig. 11) short and curved with auxiliary papilla at base. Bulla with long manubrium and mushroom-like anchor, 1.48–2.06 (1.77) in diameter.

Occurrence of copepods on various salmonids and other fishes

In the Azusa River, *S. carpionis* was found on whitespotted char (*Salvelinus leucomaenis*), brook trout (*S. fontinalis*), their natural hybrid (*S. leucomaenis* × *S. fontinalis*), and rainbow trout (*Oncorhynchus mykiss*). Similar levels of prevalence (P) and mean intensity (MI) of infection were recorded for whitespotted char (P = 48.2%, MI = 4.8, n = 409), brook trout (P = 48.7%, MI = 3.5, n = 304), and their hybrid (P = 56.0%, MI = 4.2, n = 125). Infection level on rainbow trout was low (P = 20.0%, MI = 2.0, n = 5). No amago salmon (*O. rhodurus*, n = 10) or brown trout (*Salmo trutta*, n = 166) were infected.

Two species of salmonids (whitespotted char and rainbow trout) from the Matsuo Laboratory were infected with *S. carpionis*. Infection level on whitespotted char was high (P = 80.6%, MI = 5.6, n = 1,689), but rainbow trout were

Figs. 1–11. *Salmincola carpionis*, female. Fig. 1. Entire, lateral. Fig. 2. Cephalothorax, dorsal outline. Fig. 3. First antenna, terminal segment, ventral. Fig. 4. Second antenna, entire, lateral. Fig. 5. Same, tip of exopod, lateral. Fig. 6. Same, tip of endopod, ventral. Fig. 7. Mandible, distal half, lateral. Fig. 8. First maxilla, lateral. Fig. 9. Maxilliped, entire, ventral. Fig. 10. Same, palp, ventral. Fig. 11. Same, tip of claw, ventral.



rarely infected (P = 0.8%, MI = 1.0, *n* = 752). No cherry salmon (*O. masou*, *n* = 166) were infected.

Among six species of salmonids and two other species of freshwater fishes from the Asamushi Aquarium, *S. carpionis* was found only on fishes of the genus *Salvelinus*: whitespotted char (P = 88.9%, MI = 16.6, *n* = 18), Dolly Varden (P = 9.1%, MI = 7.0, *n* = 11), Miyabe char, *S. malma miyabei* (P = 66.7%, MI = 5.1, *n* = 15), and brook trout (P = 100%, MI = 92.5, *n* = 2). Brook trout were most heavily infected, one fish harboring as many as 126 copepods. Dolly Varden were much less infected than Miyabe char, although these chars belong to the same species. No infections were observed on the following species from the tank in which infected *Salvelinus* spp. were reared: Japanese huchen (*Hucho perryi*, *n* = 17), kokanee (*O. nerka*, *n* = 60), cherry salmon (*n* = 34), rainbow trout (*n* = 6), brown trout (*n* = 3), tiger trout (brook trout × brown trout, *n* = 1), bester (*Huso huso* × *Acipenser ruthenus*, *n* = 2), threespine stickleback (*Gasterosteus aculeatus*, *n* = 51), and forktongue goby (*Chaenogobius urotaenia*, *n* = 2).

Site of infection

Salmincola carpionis was found on the walls of the buccal cavity and the skin of opercula, isthmus, snout, upper and lower jaws, near the base of fins, and on the caudal peduncle. The detailed distribution of adult female *S. carpionis* was recorded from whitespotted char (*n* = 16), Miyabe char (*n* = 10), Dolly Varden (*n* = 1), and brook trout (*n* = 2)

Table 1. Distribution of *Salmincola carpionis* on whitespotted char (*Salvelinus leucomaenis*), Miyabe char (*S. malma miyabei*), Dolly Varden (*S. malma*), and brook trout (*S. fontinalis*) from the Asamushi Aquarium, Aomori, northern Honshu.

	Whitespotted char		Miyabe char		Dolly Varden	Brook trout
Intensity per fish	1–9	≥10	1–9	≥10	1–9	≥10
No. of copepods found	33	233	34	17	7	185
Buccal cavity (total)	30(90.9)[a]	209(89.7)	32(94.1)	14(82.4)	7(100)	159(85.9)
Anterior roof	6(18.2)	68(29.2)	8(23.5)	4(23.5)	2(28.6)	19(10.3)
Posterior roof	3(9.1)	36(15.5)	2(5.9)	1(5.9)		44(23.8)
Anterior floor	16(48.5)	73(32.6)	19(55.9)	4(23.5)	1(14.3)	21(11.4)
Posterior floor	5(15.2)	32(13.7)	3(8.8)	5(29.4)	4(57.1)	75(40.5)
Opercula		10(4.3)		3(17.7)		8(4.3)
Isthmus	1(3.0)	4(1.7)	1(2.9)			
Snout	1(3.0					
Upper jaw		1(0.4)				7(3.8)
Lower jaw	1(3.0)		1(2.9)			9(4.9)
Belly						1(0.5)
Caudal peduncle		1(0.4)				
Fin bases (total)		8(3.4)				1(0.5)
Pectoral fins		6(2.6)				
Pelvic fins		1(0.4)				1(0.5)
Dorsal fin		1(0.4)				

[a]No. of copepods found (%).

from the Asamushi Aquarium (Table 1). Most (about 80–90%) copepods were found on the roof and floor of the buccal cavity. Within the buccal cavity of whitespotted char, Miyabe char, and Dolly Varden, the anterior part of the roof and floor was more heavily infected than the posterior part, including the gill arches, whereas in brook trout the posterior part of the roof and floor was more heavily infected. The second most important attachment site was the skin of the head region, such as the opercula, isthmus, snout, and upper and lower jaws. Some copepods also occurred near the base of the fins (mainly the pectoral fin, rarely the pelvic or dorsal fin). At high intensities (≥10 copepods/fish), parts other than the buccal cavity were a little more frequently infected (Table 1).

The bullae of adult copepods were deeply embedded in the host's tissues, which were often swollen. Hemorrhages were sometimes observed at the attachment sites on the roof and floor of the buccal cavity of heavily infected fish.

Occurrence of copepods related to host size and age

In the Azusa River, prevalence of infection with *S. caprionis* increased significantly with increasing length of whitespotted char and brook trout (*G*-test, $G_{adj} = 25.779$, df = 5, $p < 0.001$ for whitespotted char, $G_{adj} = 10.345$, df = 3, $p < 0.025$ for brook trout, Fig. 12). Similarly, mean intensity of infection steadily increased with increased length of whitespotted char and brook trout (ANOVA, $F = 15.984$, $p < 0.001$ for whitespotted char, $F = 3.128$, $p < 0.05$ for brook trout). There was an increase in infection levels with size of hybrid, but these differences were not statistically significant ($p > 0.05$).

Prevalence was significantly higher in larger and older whitespotted char reared at the Matsuo Laboratory (*G*-test, $G_{adj} = 285.034$, df = 4, $p < 0.001$ for size, $G_{adj} = 153.203$, df = 2, $p < 0.001$ for age, Fig. 13). Mean intensity also increased significantly with size and age of these fish (ANOVA, $F = 256.310$, $p < 0.001$ for size, $F = 78.274$, $p < 0.001$ for age).

There was a similar relationship between copepod prevalence and size of salmonids from the Asamushi Aquarium.

Seasonal occurrence of copepods

Seasonal changes in prevalence and mean intensity of *S. carpionis* infection on whitespotted char ($n = 1635$), rainbow trout ($n = 720$), and cherry salmon ($n = 150$) were monitored at the Matsuo Laboratory from June 1984 to May 1985. Water temperature used at this laboratory was almost stable, being at about 12°C.

Prevalence on age 1 whitespotted char increased sharply (Fig. 14). Prevalences on age 2 and ≥3 char were stable at a high level. Mean intensities on all age groups increased steadily. These increases in prevalence on age 1 char and mean intensity on all age groups were related to the increase in fork length of each age group of the char.

Rainbow trout were rarely infected, and there were no seasonal trends in prevalence or mean intensity. No cherry salmon were infected.

Discussion

Identification

The morphology of the copepods examined in the present study corresponds to that of *Salmincola carpionis*, a species that has a circumpolar distribution in the northern hemisphere (Kabata 1969). Although Markevich's (1956) record of *S. carpionis* (as *S. smirnovi* Markevich, 1956) from Japan has been regarded as questionable (Nagasawa et al.

Fig. 12. Prevalence (open bars) and mean intensity (hatched bars) of infection with *Salmincola carpionis* on whitespotted char (*Salvelinus leucomaenis*, top), brook trout (*S. fontinalis*, middle), and their natural hybrid (*S. leucomaenis* × *S. fontinalis*, bottom) of various sizes (5 cm length intervals) from the Azusa River, Nagano, central Honshu. Numbers above bars show sample sizes.

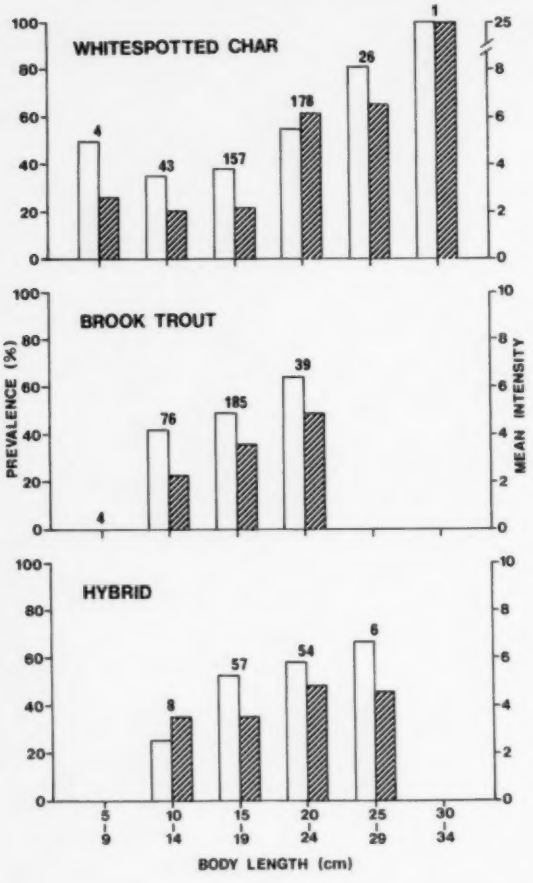
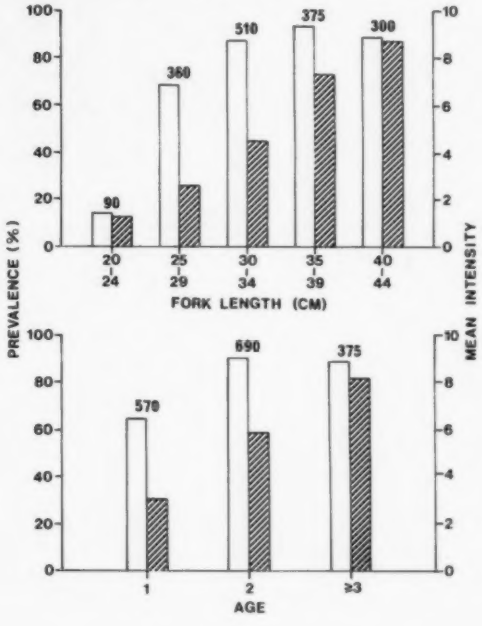


Fig. 13. Prevalence (open bars) and mean intensity (hatched bars) of infection with *Salmincola carpionis* on whitespotted char (*Salvelinus leucomaenis*) of various sizes (top, 5 cm length intervals) and ages (bottom) reared at the Iwate Prefectural Inland Fisheries Experimental Station, Iwate, northern Honshu. Numbers above bars show sample sizes.



1987), this study confirms its presence in Japan. An unidentified species of *Salmincola* reported by Kumagai (1985) from whitespotted char and rainbow trout from the Matsuo Laboratory is also assignable to *S. carpionis*.

In Far East Asia, *S. carpionis* has been reported from Sakhalin, Russia (Kabata 1969), an island north of Japan. In Japan, Yamaguti (1939) described adult female *S. falculata* (Wilson, 1908) from kokanee from Lake Penke, eastern Hokkaido (the name of the lake was probably a misspelling of Lake Panke, which is connected with Lake Penke, In the 1930's salmonids were not present in Lake Penke. However, kokanee were introduced to Lake Panke in 1937, 2 years before Yamaguti's description) (Dr. T. Awakura, Hokkaido Fish Hatchery, Eniwa, Hokkaido, personal communication; see also Motoda 1950). Based on a comprehensive review of the genus *Salmincola*, Kabata (1969) suggested that the copepods reported as *S. falculata* from Asia are referable to *S. californiensis*. However, the description of *S. falculata* given by Yamaguti (1939) corresponds to *S. carpionis* rather than to *S. californiensis*. Although the mandible of Yamaguti's specimens was equipped with eight teeth, the bulla was mushroom shaped (1.45 mm in diameter), and the exopod of the second antenna possessed two papillae near the tip. One of the morphological characters used to distinguish *S. carpionis* from *S. californiensis* is the tip of the exopod of the second antenna, which is covered with many large spines in *S. californiensis* (see Kabata 1969). The outline of the cephalothorax of Yamaguti's specimens (Yamaguti 1939: plate XLV, fig. 110) resembles that of *S. carpionis* (Fig. 2, see also Kabata 1969: figs 32, 45–46, 68–71). The total and trunk lengths of Yamaguti's specimens ranged from 4.2 to 5.2 mm and from 2.3 to 3.6 mm, respectively, which are closer to those of *S. carpionis* (4.15–6.45 mm, mean 5.02 mm; 3.00–4.70 mm, mean 3.83 mm) than those of *S. californiensis* (3.3–4.1 mm; 1.60–2.80 mm) (see Hoshina and Suenaga 1954; Kabata 1969). Moreover, Yamaguti obtained *S. falculata* from the "mouth cavity" of kokanee, the preferred attachment site of *S. carpionis*. Based on these morphological features and information on the site of attachment, the *S. falculata* described by Yamaguti (1939) is referred to *S. carpionis*. If this is true, *S. carpionis* occurs in Hokkaido as well as in Honshu.

The dimensions of *S. carpionis* vary considerably among localities (Kabata 1969). Our specimens, which measure from 3.00 to 4.70 (mean 3.83) mm in trunk length, are smaller than specimens from the Asian mainland (5.80–7.68 mm) and Greenland and Iceland (4.00–5.60 mm) but similar to those from Sakhalin (3.80–4.00 mm), suggesting that in Far East Asia, the trunk of *S. carpionis* is shorter than in other regions of the world.

Hosts

All previous host records of *S. carpionis* are from wild salmonids. Its present finding on salmonids from the Asamushi Aquarium and Matsuo Laboratory represents the first record of this parasite from fishes reared under artificial conditions. Like *S. californiensis* (see Gall et al. 1972; Hoshina and Nishimura 1976; Sutherland and Wittrock 1985), *S. carpionis* may become a pathogen of salmonids (mainly *Salvelinus* spp.) at fish farms.

This study has shown that *S. carpionis* is specific to fishes of the genus *Salvelinus*. Previously known hosts comprise five species (including one subspecies) of the genus *Salvelinus* and one species of the genus *Oncorhynchus* (*S. alpinus*, *S. alpinus alpes*, *S. malma*, *S. taimyricus*, *S. leucomaenis*, *S. fontinalis*, and *O. nerka*) (Kabata 1969, 1988). Miyabe char and rainbow trout are new hosts for *S. carpionis*, but the latter appears to be an unsuitable host because the infection level on this salmonid was low.

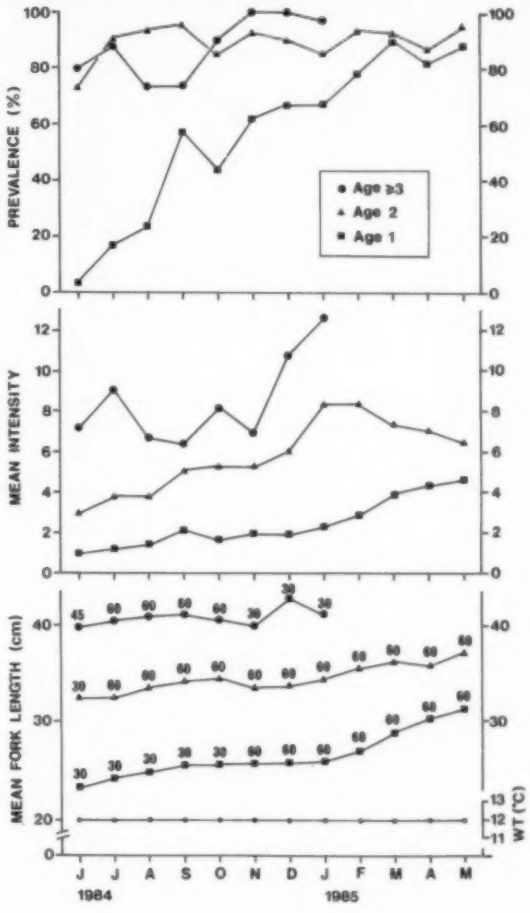
Although Yamaguti (1939) reported *S. carpionis* (as *S. falculata*) from the kokanee, this salmonid may also be an unsuitable host, since this parasite never occurred on the kokanee which we examined at the Asamushi Aquarium. Examination of adult kokanee ($n = 31$) taken in Lake Panke in October 1987 also revealed no *Salmincola* infection (K. Nagasawa, unpublished data). Whitespotted char are distributed in Lake Panke (Motoda 1950), where they may serve as hosts for *S. carpionis*.

Although Dolly Varden and Miyabe char differ only at the subspecific level, there was a marked difference in infection levels of *S. carpionis* between these two chars at the Asamushi Aquarium, Miyabe char being more frequently and heavily infected than Dolly Varden. Because both chars were reared in the same manner, this difference may be due to the parasite's preference for Miyabe char. Experimental infections would clarify this.

Site preference and effects on hosts

The preferred attachment site of adult female *S. caprionis* is the buccal cavity. Several other species of *Salmincola* also have preferred sites of infection: gills and the branchial cavity for *S. californiensis* (see Kabata and Cousens 1977; Sutherland and Wittrock 1985); fins, fin bases, and gills for *S. edwardsii* (Olsson, 1869) (see Fryer 1981; Black 1981; Black et al. 1983; Conley and Curtis 1994); gills for *S. salmoneus* (Linnaeus, 1758) (see Friend 1941); the dorsal anterior portion of the branchial rim for *S. corpulentus* (Kellicott, 1880) (see Bowen and Stedman 1990); the buccal cavity for *S. lotae* (Olsson, 1877) (see Lasse et al. 1988) and *S. stellatus* (see Nagasawa and Urawa 1991); and fin bases for *S. siscowet* (Smith, 1874) (see Anderson 1993). This indicates that larvae of

Fig. 14. Monthly changes in prevalence and mean intensity of infection with *Salmincola carpionis* on whitespotted char (*Salvelinus leucomaenis*) of various ages reared at the Iwate Prefectural Inland Fisheries Experimental Station, Iwate, northern Honshu, from June 1984 to May 1985. Numbers above the values of individual mean fork lengths of char show sample sizes. Top, prevalence; middle, mean intensity; bottom, fork length of char and water temperature (WT).



Salmincola spp. move to their preferred site after they attach to the host's body surface and before they implant the bulla (see Kabata and Cousens 1977; Poulin et al. 1991*b*; Conley and Curtis 1994).

Black et al. (1983) reported a shift in the attachment site of *S. edwardsii* with increased intensity of infection, the major attachment site of this parasite changing from the adipose fin at low intensities to the dorsal fin at high intensities. In this study, we found only slight displacement in the attachment site of *S. carpionis* with increased intensity of infection. This is probably because the walls of the buccal cavity usually have enough space to accommodate relatively high numbers of *S. carpionis*.

Salmincola californiensis has been reported to reduce the resistance of brook trout and rainbow trout to environmental stress or high temperature and low dissolved oxygen (Vaughan and Coble 1975; Sutherland and Wittrock 1985) and may reduce the fecundity of rainbow trout (Gall et al. 1972). However, no information is available on the effects of *S. carpionis* on its host. During this study, hemorrhages were sometimes observed at the attachment sites, and it is possible that these may lead to secondary bacterial infections. Moreover, because the walls of the buccal cavity of very heavily infected fish were covered with many copepods, such fish may be stressed and reduce their feeding activity. Nagasawa et al. (1994) reported that the appetite of Japanese huchen infected with *S. stellatus* was reduced.

Age-related and seasonal occurrence of copepods

Prevalence and mean intensity of infection with *S. carpionis* increased with host length and age. Similar results were obtained for *S. corpulentus* on bloater (*Coregonus hoyi*) from Lake Huron by Bowen and Stedman (1990). This may be explained by the recent work of Poulin et al. (1991*a*), who have experimentally shown that the size of brook trout influences the probability of acquiring larval *S. edwardsii*, large fish acquiring more copepods than small fish during the same duration of exposure.

There were no seasonal changes in prevalence or mean intensity of *S. carpionis* on whitespotted char at the Matsuo Laboratory. Prevalence on age 1 fish and mean intensities on fish of all age groups increased with increasing length of the fish. These results suggest that infection occurs throughout the year. Although we did not examine the maturity of female copepods, such a continuous infection may have resulted from continuous egg production under the condition of constant water temperature (about 12°C) for rearing salmonids.

In conclusion, *S. carpionis* was rediscovered in central and northern Honshu, Japan, where this parasite occurred on species of *Salvelinus* (whitespotted char, brook trout, their natural hybrid, Dolly Varden, Miyabe char) and rarely on rainbow trout. The copepods were found on both wild and reared fishes. The preferred attachment site was the buccal cavity, but at high intensities copepods were a little more frequently found on other parts. Prevalence and mean intensity of infection increased with increasing size and age of the fish. At a constant water temperature, there were no seasonal changes in prevalence and mean intensity of copepod infection. The copepods that were previously described as *Salmincola falculata* and later regarded as *S. californiensis* from kokanee from Hokkaido, northern Japan, are assigned to *S. carpionis*.

Acknowledgements

We thank the staff of the Asamushi Aquarium and Messrs. S. Yamauchi and S. Watanabe of the Iwate Prefectural Inland Fisheries Experimental Station for their assistance with sampling. Thanks also go to Dr. T. Awakura of the Hokkaido Fish Hatchery for information on salmonids in Lakes Penke and Panke, Hokkaido. Dr. Z. Kabata of the Pacific Biological Station, Nanaimo, Canada, and Dr. J.R. Arthur of the Maurice Lamontagne Institute, Mont-Joli, Canada, reviewed the manuscript.

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Parasitological evidence for specialized foraging behavior in lake-resident Arctic char (*Salvelinus alpinus*)

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Abstract: Analyses of statistical associations between the stomach contents and endoparasites of Arctic char, *Salvelinus alpinus*, from a small (9 ha) lake in northern Quebec revealed that food items found in fish stomachs at the time of capture frequently consisted of intermediate hosts for the parasites infecting the fish. Thus the stomach contents of Arctic char infected by *Diphyllobothrium ditremum*, *D. dendriticum*, and *Eubothrium salvelini* tended to include copepods, while fish infected by the digenean *Crepidostomum farionis* more frequently contained insect larvae (ephemeropterans) and fish infected by the acanthocephalan *Echinorhynchus lateralis* most often had amphipods in their stomachs. Moreover, strong quantitative associations were evident between parasites utilizing intermediate hosts from either the benthic or the limnetic zone of the lake. This suggests that some degree of persistent feeding specialization was present among members of the Arctic char population over an extended period of time, with individual fish predominantly feeding upon prey organisms in either limnetic or benthic habitats. In this manner an allopatric Arctic char population may function analogously to more diverse fish communities, where specialist foraging behavior is developed to most efficiently exploit the food web.

Résumé : L'analyse des associations statistiques entre les endoparasites qui infectent l'omble chevalier, *Salvelinus fontinalis*, et les contenus stomacaux d'ombles prélevés dans un petit lac (9 ha) du nord du Québec, montre que ces poissons se nourrissaient fréquemment, au moment de leur capture, d'hôtes intermédiaires des parasites. Dans le contenu stomacal des ombles infectés par *Diphyllobothrium ditremum*, *D. dendriticum* et *Eubothrium salvelini*, on trouvait des copépodes en abondance tandis que dans celui des ombles infectés par le digénien *Crepidostomum farionis*, on trouvait plus fréquemment des larves d'insectes (éphéméroptères). Chez les ombles infectés par l'acanthocéphale *Echinorhynchus lateralis*, on trouvait le plus souvent des amphipodes dans le contenu stomacal. De plus, il ressortait de fortes associations quantitatives entre les parasites qui utilisaient des hôtes intermédiaires benthiques et ceux qui utilisaient des hôtes intermédiaires limniques. On peut penser qu'il existait une certaine spécialisation de l'alimentation au sein de la population d'ombles, et qui persistait un certain temps, certains se nourrissant surtout de proies de la zone limnique, d'autres de proies de la zone benthique. Ainsi, une population allopatrique d'ombles chevaliers peut se comporter de la même façon que des communautés plus diversifiées, chez qui l'alimentation se spécialise pour permettre de tirer le meilleur parti possible du réseau trophique.

[Traduit par la Rédaction]

The feeding habits of Arctic char, *Salvelinus alpinus*, have been examined carefully on a seasonal basis in a variety of lake habitats in Europe and North America. Food items differ according to fish size and age, the availability of suitable prey, and the presence or absence of other interacting fish species (Nilsson 1955; Hunter 1970; Johnson 1980; Fraser and Power 1984). Customarily, field studies of this nature have been based on periodic examinations of fish stomach content samples. However, such samples in themselves give information only on the most recently

Received March 1, 1994. Accepted March 16, 1995.
J12850

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consumed foods of each individual fish over a period of a few days at most. Ideally, to assess the foraging behavior of a particular fish over a more extended period, that individual must be sampled or observed repeatedly, a process that is impractical for members of any sizeable lake-resident population, although technically feasible for salmonids dwelling in small ponds or streams (Bryan and Larkin 1972; Bachman 1984; Grant and Noakes 1987). As an alternative to repeated sampling, this paper demonstrates that quantitative information on salmonid endoparasites may be usefully analysed to indicate whether or not feeding specialization occurs on an individual basis in lake-resident populations.

In small lakes at higher elevations in the Ungava region of northern Quebec, Arctic char often occur as the only salmonid species. Under these circumstances they consume a variety of food items, including benthic organisms, zooplankton, and *Gasterosteus aculeatus*, the threespine stickleback (Fraser and Power 1984; Bérubé and Curtis 1986). Within samples of similarly sized Arctic char from the lakes, stomach contents contain a predominance of prey originating from the limnetic or benthic components of the ecosystem. One may hypothesize that observed differences among fish stomach contents are due to some degree of feeding specialization lasting for a period of at least several weeks. An alternate hypothesis is that apparent differences in diet reflect short-term foraging activities that depend largely upon prey encountered by each individual Arctic char on a daily basis, with all fish within a size group opportunistically utilizing the complete range of food sources available to them.

We have tested these hypotheses by examining associations between Arctic char stomach contents and endoparasites. Arctic char become infected by certain parasites (*Diphyllobothrium ditremum* (Creplin, 1825), *D. dendriticum* (Nitzsch, 1824), *Eubothrium salvelini* (Schrank, 1790)) when they feed on copepods or sticklebacks in the limnetic zone of a lake and by other parasites (*Crepidostomum farionis* (Müller, 1780), *Echinorhynchus lateralis* (Leidy, 1851)), when they feed in the benthic zone (Bérubé and Curtis 1986). All these parasites persist in the fish hosts for at least several months after initial acquisition. If individual Arctic char tend to specialize in their foraging behavior over an appreciable time interval, there should be statistically significant associations between the parasite species infecting them and the occurrences in their stomachs of prey which serve as intermediate hosts for these parasites. No such associations should occur if Arctic char feed opportunistically.

In this context, it is pertinent to describe the food web pathways by which common endoparasites are transmitted to Arctic char. Hoffman (1967) provides information on intermediate hosts of these salmonid parasites. *Diphyllobothrium ditremum* and *D. dendriticum* occur as procercoids in the copepod *Cyclops scutifer* and develop as plerocercoids in the visceral organs of Arctic char feeding on zooplankton. Both *Diphyllobothrium* species are also acquired by Arctic char consuming threespine and ninespine sticklebacks (*Gasterosteus aculeatus*) by fish-to-fish transmission of the plerocercoids (Curtis 1984; Bérubé and Curtis 1986). *Eubothrium salvelini*, which occurs as an adult cestode in the anterior intestine of Arctic char, is acquired by fish foraging on copepods. Arctic char acquire intestinal infections of the adult trematode *Crepidostomum farionis* after consuming ephemeropterans (mayfly larvae) or amphipods containing the metacercarial stage of the parasite. The acanthocephalan *Echinorhynchus lateralis* infects fish that feed upon benthic amphipods harboring acanthella larvae, and the adult form develops in the posterior intestine of the host fish.

Materials and methods

Arctic char were sampled by gill nets (25, 38, or 63 mm mesh) in early (27 June – 5 July) and late summer (27 August – 12 September) 1983 at Lac Kitturiak (57°47′N, 69°28′W), located about 70 km southwest of Kuujjuaq, Quebec. Basic information on lake characteristics, Arctic char population structure, and *Diphyllobothrium* spp. transmission has been documented by Bérubé and Curtis (1986). The lake, which has a surface area of 9 ha and a maximum depth of 13 m, contains *S. alpinus* and *G. aculeatus* as the only fish species. The Arctic char grow slowly and very few attain a fork length exceeding 220 mm. Piscivorous feeding is found in all size classes over 120 mm, and fish from 120 to 200 mm are able to exploit all other food sources available in the lake (Bérubé and Curtis 1986).

After capture, the Arctic char were eviscerated and the stomach contents and intestinal tract, including pyloric caeca, were preserved in 4% buffered formaldehyde. The stomach and remaining visceral organs were digested in an HCl–pepsin solution (Meyer and Vik 1963) at 30°C for recovery of *Diphyllobothrium* spp. plerocercoids and other endoparasites; the digest residue with parasites was then preserved in 4% formaldehyde. Only Arctic char with identifiable stomach contents were used for the food–parasite association data set. Food items in stomachs were recorded on a presence–absence basis, while all parasites were enumerated on an ordinal scale (0, 1–5, 6–20, 21–50, 51+), resulting in five categories of abundance. In addition, precise counts of abundance were obtained for *D. ditremum*, *D. dendriticum*, and *Echinorhynchus lateralis*. Statistical analyses were applied only to Arctic char measuring between 120 and 200 mm in fork length to ensure that we were dealing with a reasonably homogeneous group of fish with the potential to exploit the same types of prey organisms. Within this size group, length and age are not significantly associated with food selectivity (see Bérubé and Curtis 1986). Prey organisms in Arctic char stomachs were grouped into general categories to facilitate statistical analyses. All sticklebacks were *G. aculeatus*. Copepods were *Cyclops scutifer*, *Leptodiaptomus minutus*, and *Epischura lacustris*, whereas cladocerans were *Holopedium gibberum*, *Bosmina longirostris*, and *Daphnia longiremis*. *Pisidium* sp. was the only pelecypod. Amphipods and insects were not identified to species. The categories "insect larvae" and "insect pupae" refer to life-cycle stages of insects, primarily dipterans, tricopterans, and ephemeropterans. "Insect adults" were principally non-aquatic stages of dipterans, trichopterans, ephemeropterans, and coleopterans. If Arctic char stomach contents included sticklebacks

Table 1. Summary of parasite prevalences (%), occurrence of food items (%), and host data for Arctic char collected from Lac Kitturiak, Quebec in 1983.

	27 June – 5 July	27 August – 12 September	Totals
Parasites			
Diphyllobothrium ditremum	68.9	80.1	74.9
D. dendriticum	4.2	22.1	13.7
Eubothrium salvelini	40.3	51.5	46.3
Crepidostomum farionis	88.2	51.5	68.6
Echinorhynchus lateralis	86.6	75.4	80.6
Food items			
Threespine sticklebacks	12.6	11.0	11.8
Copepods	6.7	34.6	21.6
Insect larvae	73.9	19.9	45.1
Insect pupae	61.3	58.8	60.0
Insect adults	6.7	50.0	29.8
Amphipods	23.5	5.1	13.7
Pelecypods	10.9	3.7	7.1
Host data			
Fish mean length (mm)	149	140	144
Range of length	120–199	120–197	120–199
Mean weight (g)	33	26	29
Range of weight	14–76	15–75	14–76
Mean age (years)	6.6	5.8	6.2
Sample size (N)	119	136	255

and copepods, the copepods were excluded from the data set because they could have originated as undigested stickleback prey.

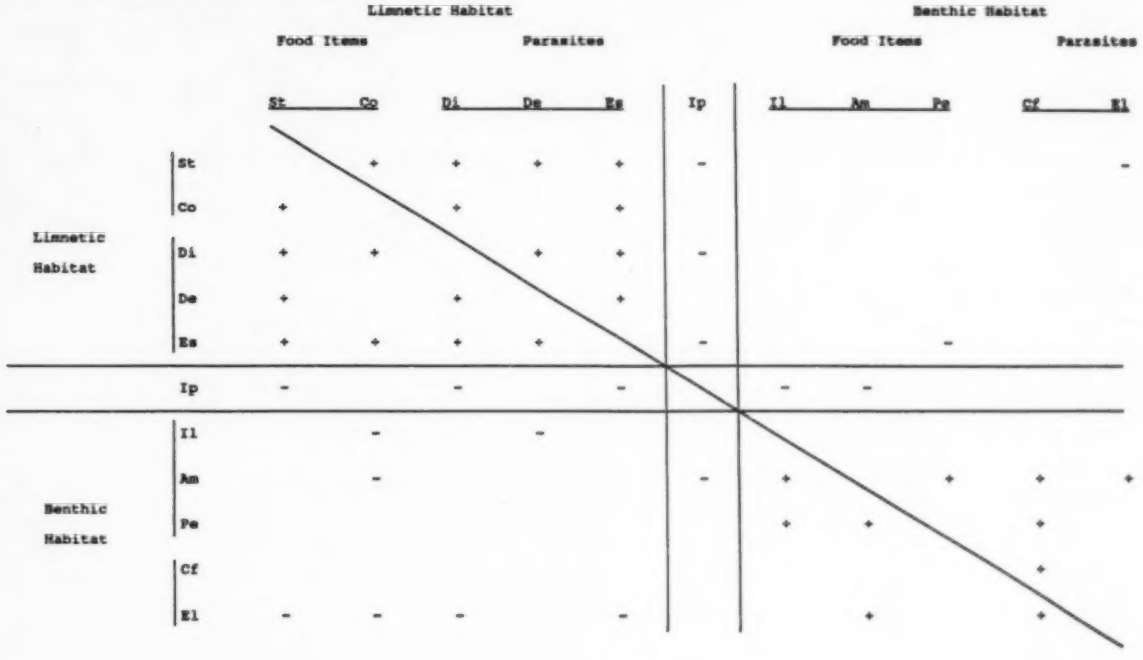
Associations between food items (presence or absence) and parasites (five scales of abundance) were analysed separately in early (June–July) and late (August–September) summer by means of 2×5 contingency tables, with statistically significant differences evaluated by G-test (Sokal and Rohlf 1981). Tables for which the null–null (0×0) cell contained 80% or more of the cross-tabulations were excluded from significance testing. G-tests were similarly performed on food–food (2×2) and parasite–parasite (5×5) contingency tables. To further quantify these relationships, parasite prevalence was then calculated for fish samples categorized according to the occurrence or non-occurrence of each food type and parasite grouping. Statistical significance of the differences in prevalence was determined by G-test. Finally, abundance for *D. ditremum*, *D. dendriticum*, and *Echinorhynchus lateralis* was determined for fish grouped by food type and parasite co-occurrence, and statistically significant differences measured by Student's t-test on the log-transformed abundance data ($\log_{10}(n + 1)$).

Results

Marked seasonal differences in Arctic char stomach contents were evident, with benthic foods (e.g., insect larvae, amphipods) relatively important in early summer, and limnetic foods (e.g., copepods) more prominent in late summer (Table 1, see also Bérubé and Curtis 1986). Sticklebacks and insect pupae were relatively unchanged in their percentage of occurrence during both sampling periods, and insect adults were only of any real importance as prey items in late summer. Prevalences of the parasites *D. ditremum*, *D. dendriticum*, and *E. salvelini* increased between early and late summer, while prevalences of *C. farionis* and *E. lateralis* decreased. Except for *E. salvelini*, all changes in parasite prevalence were significant ($P < 0.05$).

Figure 1 provides a summary of results for the statistical tests of associations between parasites and fish food items during the early and late summer sampling periods. Many food–parasite associations were statistically significant ($P < 0.05$) in a manner that positively related the parasites to their intermediate hosts (e.g., *D. ditremum* with copepods and threespine sticklebacks); conversely, significant negative associations appeared between parasites and food items that did not serve as intermediate hosts (e.g., *E. lateralis* with copepods and threespine sticklebacks). Significant associations between food items were positive for those occurring in the same lake habitat (e.g., amphipods and pelecypods), but negative for those from different habitats (e.g., amphipods and copepods). Among parasite species, significant positive associations were evident for those acquired by fish feeding on intermediate host

Fig. 1. Statistically significant associations (G-test $P < 0.05$) of food–parasite, food–food, and parasite–parasite contingency tables for Arctic char samples collected 27 June – 5 July 1983 (above the diagonal line) and 27 August – 12 September 1983 (below the diagonal). Statistical significance tested by 2 × 5 contingency tables and G-test. Significant ($P < 0.05$) positive associations indicated by a plus sign (+); significant ($P < 0.05$) negative associations indicated by a minus sign (−). St, threespine sticklebacks (*Gasterosteus aculeatus*); Co, copepods; Ip, insect pupae; Il, insect larvae; Am, amphipods; Pe, pelecypods; Di, *Diphyllobothrium ditremum*; De, *D. dendriticum*; Es, *Eubothrium salvelini*; Cf, *Crepidostomum farionis*; El, *Echinorhynchus lateralis*.



organisms occupying the same type of lake habitat (e.g., *E. lateralis* and *C. Farionis*, transmitted by amphipods and ephemeropterans), while negative associations occurred among parasites acquired by fish feeding on intermediate hosts occupying very different zones in the lake (e.g., *E. lateralis* and *D. ditremum*, transmitted by amphipods and copepods, respectively). Within the association matrix summarized by Fig. 1 statistically significant positive associations were more numerous than significant negative associations (28 vs. 17). Nonsense associations (statistically significant associations inconsistent with the patterns described above) were conspicuously absent.

Parasite prevalences, calculated for fish samples categorized by the presence or absence of particular food items, can be used to further quantify differences in parasite occurrence for Arctic char with different feeding habits (Table 2). For example, with reference to the total sample material (early summer + late summer combined), 96% of fish with copepods in their stomachs were infected by *D. ditremum* compared with only 69% of those not containing copepods; conversely, only 44% of the fish with copepods in their stomachs were infected by *C. farionis*, while 76% of those not containing copepods harbored the parasite. The parasite by parasite comparisons in Table 2 reveal similar patterns: 92% of all fish infected by *E. salvelini* were also infected by *D. ditremum*, while only 61% of the fish not infected by *E. salvelini* harbored *D. ditremum*; conversely, 58% of the fish infected by *E. salvelini* were also infected by *C. farionis*, while 78% of those not containing *E. salvelini* harbored *C. farionis*.

Such relationships are additionally refined by reference to abundance data available for *D. ditremum*, *D. dendriticum*, and *E. lateralis* (Table 3). For example, the abundance of *D. ditremum* was more than three times higher in fish feeding on copepods than in those not feeding on copepods (15.6 vs. 4.9). Conversely, *E. lateralis* was less than half as abundant in fish feeding on copepods as in those not so feeding (3.2 vs. 7.9). Results of parasite by parasite comparisons given in Table 3 further confirm these trends: *D. ditremum* is six times more abundant in fish infected by *E. salvelini* than in those uninfected (15.6 vs. 2.6); conversely, *E. lateralis* is only about one-third as abundant in fish infected by *E. salvelini* as in uninfected fish (3.9 vs. 10.0).

Discussion

Results of the association analyses support the hypothesis that individual Arctic char in this small northern lake specialize in their feeding behavior over appreciable periods

Table 2. Parasite prevalences (%) for Arctic char grouped according to presence (+) or absence (−) of food items in stomach contents, or presence–absence of co-occurring parasites.

	Diphyllobothrium ditremum			*Diphyllobothrium dendriticum*			*Eubothrium salvelini*			*Crepidostomum farionis*			*Echinorhynchus lateralis*		
	June	August	Total	June	August	Total	June	August	Total	June	August	Total	June	August	Total
Stickleback															
+	73.3	100.0 *	87.6	13.3	60.0 *	36.7 *	66.7 *	93.3 *	80.0 *	73.3	46.7	60.0	73.3	73.3	73.3
−	68.3	77.7	73.3	2.9	17.4	10.7	36.5	46.3	41.8	90.4	52.1	69.8	88.4	76.0	81.8
Copepod															
+	100.0 *	95.7 *	96.4 *	0.0	25.5	21.8	50.0	76.6 *	72.7 *	75.0	38.3 *	43.6 *	87.5	59.6 *	63.6 *
−	66.7	71.9	69.0	4.5	20.0	11.5	39.6	38.2	39.0	89.2	58.2	75.5	86.5	84.3	85.5
Insect pupa															
+	63.0	82.5	73.2	1.4 *	16.3 *	9.2 *	30.1 *	43.7 *	37.3 *	91.8	48.7	69.3 *	87.7	73.7	80.4
−	38.3	76.8	77.5	8.7	30.4	20.6	56.5	62.5	59.8	82.6	55.4	67.6	84.8	78.6	81.4
Insect larva															
+	69.3	81.5	72.2	5.7	7.4 *	6.1 *	39.8	48.1	41.7	88.6	63.0	82.6 *	89.8	74.1	86.1 *
−	67.7	79.8	77.1	0.0	25.7	20.0	41.9	52.3	50.0	87.1	48.6	57.1	77.4	76.1	76.4
Amphipod															
+	64.3	57.1	62.9	3.6	0.0 *	2.9 *	39.3	28.6	37.1	100.0 *	85.7 *	97.1 *	96.4 *	100.0 *	97.1 *
−	70.3	81.4	76.8	4.4	23.2	15.5	40.7	52.7	47.7	84.6	49.6	64.1	83.5	74.4	78.2
Pelecypod															
+	76.9	80.0	77.8	7.7	20.0	11.1	30.8	40.0	33.3	100.0	100.0 *	100.0 *	84.6	80.0	83.3
−	67.9	80.1	74.7	3.8	22.1	13.9	41.5	51.9	47.3	86.8	49.6	66.2	86.8	75.6	80.6
D. ditremum															
+				6.0 *	27.5 *	18.3 *	51.2 *	60.6 *	56.5 *	86.6	50.5	66.0	84.2	70.6 *	76.4 *
−				0.0	0.0	0.0	16.0	14.8	15.6	91.9	55.6	76.6	91.9	93.6	93.8
D. dendriticum															
+	100.0 *	100.0 *	100.0 *				100.0 *	76.7 *	80.0 *	60.0	40.0	42.9 *	80.0	70.0	71.4
−	67.5	74.5	70.9				37.7	44.3	40.9	89.5	54.7	72.7	86.8	77.4	82.3
E. salvelini															
+	87.5 *	94.3 *	91.5 *	10.4 *	32.9 *	23.7 *				81.3 *	41.4 *	57.6 *	79.2 *	61.4 *	68.6 *
−	56.3	65.2	60.6	0.0	10.6	5.1				93.0	62.1	78.1	91.6	90.9	91.2

Table 2 (*concluded*).

	Diphyllobothrium ditremum			*Diphyllobothrium dendriticum*			*Eubothrium salvelini*			*Crepidostomum farionis*			*Echinorhynchus lateralis*		
	June	August	Total	June	August	Total	June	August	Total	June	August	Total	June	August	Total
C. farionis															
+	67.6	78.6	72.0	2.9	17.1	8.6 *	37.1 *	41.4 *	38.9 *				86.7	88.6 *	87.4 *
−	78.6	81.8	81.3	14.3	27.3	25.0	64.3	62.1	62.5				85.7	62.1	66.3
E. lateralis															
+	67.0	74.8 *	70.9 *	3.9	20.4	12.1	39.6 *	41.8	39.3 *	84.4	60.2 *	74.3 *			
−	81.3	97.0	91.8	6.3	27.3	20.4	62.5	81.8	75.5	87.5	24.2	44.9			

Note: Significant differences between prevalences are indicated by an asterisk (*), determined by G-test ($P < 0.05$).

of time, rather than feeding opportunistically throughout the open water season. It appears that Arctic char in Lac Kitturiak tended to concentrate their feeding efforts upon particular types of prey organisms, with some fish favoring limnetic feeding on copepods or threespine sticklebacks, whereas others tended towards benthic feeding on insect larvae, amphipods, and pelecypods. This interpretation is supported by statistically significant negative and positive associations between food items and parasites and by complementary findings on associations among the various parasite species. It is further verified by the observed differences in prevalence and abundance values for parasites in fish categorized by food or parasite co-occurrence. If the Arctic char sampled had been entirely opportunistic in feeding behavior, randomly exploiting all prey types available to them in the lake, it would be unlikely by chance alone to find the strong quantitative patterns of association reported here.

Among the food items of Arctic char, only insect pupae and insect larvae failed to demonstrate any positive associations with other foods or parasites. In addition, insect pupae (mostly chironomids) were negatively associated with both limnetic and benthic elements of the matrix (i.e., threespine sticklebacks, insect larvae, amphipods, *D. ditremum*, *E. salvelini*). One may account for this seemingly anomalous result by the observation that insect pupae can be captured by fish either on the bottom or during their upward movements through the water column prior to emergence. Thus insect pupae are potential prey for both limnetic and benthic feeding Arctic char. It is also possible that all Arctic char in Lac Kitturiak, regardless of feeding preference, respond to particularly intense periods of insect emergence and high prey availability.

Some questions remain as to the exact meaning of the results of association analyses in terms of the feeding behavior of individual fish. Statistical evidence from the food–parasite and parasite–parasite associations clearly points towards individuals tending to feed more in either the limnetic or the benthic zone of the lake. Yet, the mix of food items and parasites in many fish indicates that they at times do forage in both zones. Seasonal changes in food availability and feeding probably account for this pattern, as Arctic char in Lac Kitturiak are known to shift their feeding from the benthic zone to the limnetic zone as the open water season progresses (Bérubé and Curtis 1986). The extent to which individual fish can accommodate such changes may influence their ultimate determination as predominantly "limnetic" or "benthic" feeders. This situation may be somewhat analogous to that for young-of-the-year brook trout (*Salvelinus fontinalis*), which in running water tend to behave either as active swimmers ("movers") or sit and wait foragers ("stayers") (Grant and Noakes 1987). Such specialization in feeding behavior of salmonids can generally be attributed to the influence of previous foraging experience (Ringler 1979; Allan 1981).

Arctic char in Scandinavia and Iceland are polymorphic and occur as several types, each differing from the others with regard to genetics, spawning habitat, and feeding behavior (Klemetsen and Grotnes 1975; Nyman et al. 1981; Frandsen et al. 1989). Henricson and Nyman (1976)

Table 3. Parasite abundances for Arctic char grouped according to presence (+) or absence (−) of particular food items in stomach contents, or presence–absence of co-occurring parasites.

	Diphyllobothrium ditremum			*Diphyllobothrium dendriticum*			*Echinorhynchus lateralis*		
	June	August	Total	June	August	Total	June	August	Total
Stickleback									
+	19.0 *	36.9 *	26.5 *	0.3 *	1.0 *	0.6 *	3.3 *	5.5	4.2
−	4.5	5.8	5.2	0.0	0.2	0.1	11.7	4.2	6.9
Copepod									
+	25.6 *	14.3 *	15.6 *	0.0	0.2	0.2	10.8	2.5 *	3.2 *
−	4.8	4.9	4.9	0.1	0.3	0.2	10.0	5.8	7.9
Insect pupa									
+	3.6 *	6.1	4.7 *	0.0	0.1 *	0.1 *	10.7	4.2	6.7
−	10.2	9.2	9.7	0.2	0.4	0.3	9.1	4.5	6.3
Insect larva									
+	5.0	7.6	5.5	0.1	0.2	0.1	9.9	4.0	8.1 *
−	7.0	7.1	8.7	0.0	0.3	0.2	10.4	4.4	5.4
Amphipod									
+	5.0	6.0	5.2	0.0	0.0	0.0 *	15.9 *	23.7 *	17.3 *
−	5.6	7.3	6.6	0.1	0.3	0.2	8.7	3.9	5.5
Pelecypod									
+	9.3	7.1	8.6	0.2	0.5	0.3	8.2	5.8	7.5
−	5.1	7.2	6.2	0.1	0.2	0.2	10.3	4.3	6.5
Diphyllobothrium ditremum									
+				0.1	0.3 *	0.2 *	8.3 *	3.9	5.5 *
−				0.0	0.0	0.0	15.1	6.5	10.8
Diphyllobothrium dendriticum									
+	46.3 *	22.6 *	24.7 *				3.4	3.8	3.7 *
−	4.9	5.1	5.0				10.5	4.5	7.1
Eubothrium salvelini									
+	13.7 *	17.1 *	15.6 *	0.2 *	0.4 *	0.3 *	8.5 *	2.6 *	3.9 *
−	4.9	2.6	2.6	0.0	0.1	0.0	13.2	7.1	10.0
Crepidostomum farionis									
+	4.9	5.5 *	5.1 *	0.0	0.2	0.1 *	10.6	7.4 *	9.2 *
−	12.0	9.6	9.9	0.3	0.3	0.3	6.8	3.2	2.8
Echinorhynchus lateralis									
+	4.8 *	5.4 *	5.1 *	0.1	0.2	0.1			
−	12.3	16.7	15.1	0.1	0.3	0.2			

Note: Significant differences between abundances are indicated by an asterisk (*), determined by *t*-test ($P < 0.05$) on $\log_{10}$ transformed parasite abundance data.

have shown that Arctic char types living sympatrically also differ in parasite prevalence. For example, at a lake in northern Sweden, they report finding *D. ditremum* and *D. dendriticum* more prevalent in "pelagic" Arctic char while *Crepidostomum metoecus* (Braun, 1900) and *Cyathocephalus truncatus* (Pallas, 1781), a cestode using *Gammarus* as an intermediate host, are more prevalent in "benthic" Arctic char. Frandsen et al. (1989) describe quantitative differences between the endoparasite associations of five Arctic char morphs in Thingvallavatn, Iceland, again relating these to habitat and feeding preferences. However, the phenomenon of several Arctic char types occurring as nonmigratory forms in a single lake has not been previously reported in North America, and the results from Lac Kitturiak can be more easily ascribed to persistent patterns of individual foraging behavior than by postulating the existence of two or more genetically distinct morphs.

Analyses of Arctic char parasites have often been utilized in the past to gain information about fish foraging behavior and to separate lake-resident from migratory forms (e.g., Eddy and Lankester 1978; Dick and Belosevic 1981). Yet, no previous study has statistically linked fish stomach contents to parasites in the manner described herein, although this would have likely occurred if resident Arctic char populations commonly exhibited strong relationships between endoparasites and stomach contents at the time of capture. Moreover, when data from nearby Lac Milugiak, a more productive lake (Bérubé and Curtis 1986), were submitted to an identical statistical treatment, few significant food–parasite associations emerged from the analysis. It is conceivable that the poor feeding conditions in Lac Kitturiak were an important factor in inducing the comparatively high degree of feeding specialization found among members of its Arctic char population, but if so, this would contradict theoretical models suggesting fish respond to low levels of prey availability by broadening their diets (Dill 1983). Further investigations are necessary to discover the extent of occurrence of individual specialization within allopatric lake-resident Arctic char populations, and the factors inducing this behavior.

Under the circumstances described herein for the Arctic char of Lac Kitturiak, it appears that this single salmonid population is able to display the variety of foraging behaviors characteristic of a more diverse fish community comprised of several species, with each specializing to exploit different prey organisms. In this way, utilization of lake resources by the allopatric Arctic char population may be optimized.

Acknowledgements

The technical assistance of Antoine Morin during field work is gratefully acknowledged. We also thank J.C. Holmes, L. Margolis, N.H. Ringler, and V.S. Kennedy for comments on an earlier version of this manuscript. Financial support was provided by grants from the Natural Sciences and Engineering Research Council of Canada and Fonds pour la Formation de Chercheurs et l'aide à la Recherche (Québec), and supplementary logistic funds were made available through the Northern Scientific Training Program of Indian and Northern Affairs Canada.

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Parasites as biological indicators of stocks of Atlantic cod (*Gadus morhua*) off Newfoundland, Canada

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Abstract: Atlantic cod (*Gadus morhua*) were collected from six locations on the continental shelf off Newfoundland, Canada, including one inshore area and examined for parasites from 1981 to 1983. The protozoan *Loma branchialis* was more prevalent in North Atlantic Fisheries Organization (NAFO) areas 3Pn-4R (Gulf of St. Lawrence) and 2J-3K (Labrador), whereas the acanthocephalan *Echinorhynchus gadi* was more abundant in the latter than in other locations. Similarly, the prevalence and (or) abundance of the fillet-inhabiting larval nematode *Pseudoterranova decipiens* and the blood-feeding copepod *Lernaeocera branchialis* were significantly greater in fish from 3Ps (St. Pierre Bank) and 3Pn-4R than from all adjacent areas. Gastrointestinal ascaridoid nematodes were more abundant in 3M (Flemish Cap) cod than in other localities. No differences in the prevalence and abundance of *E. gadi* were detected in samples in relation to sex or size class. Paired comparisons of transformed data for the different parasite taxa revealed that there were more significant differences than similarities in cod sampled from adjacent NAFO divisions. These observations based on selected parasites (*Loma branchialis*, *Trypanosoma murmanensis*, *Myxidium gadi*, *P. decipiens*, gastrointestinal ascaridoid nematodes, *E. gadi*, and *Lernaeocera branchialis*) support the view that discrete stocks of cod inhabit the Newfoundland–Labrador continental shelf.

Résumé : De 1981 à 1983, nous avons prélevé des morues franches (*Gadus morhua*) en six endroits du plateau continental au large de Terre-Neuve (Canada), dont une zone côtière, et relevé les parasites qu'elles renfermaient. Par rapport aux autres zones de l'Organisation des pêches de l'Atlantique Nord-Ouest (OPANO), le protozoaire *Loma branchialis* était plus prévalent dans les zones 3Pn-4R (golfe du Saint-Laurent) et 2J-3K (Labrador), tandis que l'acanthocéphale *Echinorhynchus gadi* était plus abondant dans cette dernière zone. De même, la prévalence et (ou) l'abondance des larves du nématode *Pseudoterranova decipiens*, vivant dans les muscles du poisson, et du copépode hématophage *Lernaeocera branchialis* étaient significativement plus élevées dans les morues des zones 3Ps (banc de Saint-Pierre) et 3Pn-4R que dans celles de toutes les zones adjacentes. Les nématodes ascaridoïdes gastro-intestinaux étaient plus abondants dans les spécimens provenant de la zone 3M (Bonnet flamand) que dans ceux des autres endroits. Nous n'avons relevé dans les échantillons aucune différence qui soit reliée au sexe ou à la classe de taille, quant à la prévalence ou à l'abondance de l'*E. gadi*. Une comparaison par paires des données transformées relatives aux divers parasites a révélé qu'entre les morues provenant de divisions adjacentes de l'OPANO, les différences étaient plus significatives que les ressemblances. Ces observations, limitées à certains parasites (*Loma branchialis*, *Trypanosoma murmanensis*, *Myxidium gadi*, *P. decipiens*, nématodes ascaridoïdes gastro-intestinaux, *E. gadi* et *Lernaeocera branchialis*), appuient l'opinion selon laquelle plusieurs stocks distincts de morue vivent dans les eaux du plateau continental de Terre-Neuve et du Labrador.

[Traduit par la Rédaction]

Several methods have been employed in recent years to distinguish stocks of fish. Fish that share a common gene pool and are isolated during the reproductive period from other conspecific groups constitute a stock (Cross and Payne 1978). Tagging and migration studies (Templeman and Fleming 1962), meristic characters (Templeman 1962), parasites (Templeman et al. 1957, 1976; Khan et al. 1980), and serological methods (hemoglobin polymorphism and blood serum transferrins) (Cross and Payne 1978) have been useful in delineating stocks of Atlantic cod, *Gadus morhua*, inhabiting the Labrador–Newfoundland area in the northwestern Atlantic Ocean. These studies suggest that at least five stocks of cod inhabit this area. These consist of a Labrador–east Newfoundland stock complex inhabiting the North Atlantic Fisheries Organization (NAFO) divisions 2J, 3K, and 3L; another on the Grand Banks in NAFO divisions 3N and 3O; a third located on the

Received April 15, 1994. Accepted April 27, 1995.
J12889

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Flemish Cap (division 3M); a fourth on the south coast of Newfoundland in the vicinity of St. Pierre and Miquelon Islands (subdivision 3Ps); and a fifth that resides in winter on the southwestern coast (subdivision 3Pn), and occurs in summer along the western coast of the island (division 4R). Each stock has a distinct spawning area and spawns at a different time from the adjacent stock (Templeman 1974). Once the fish spawn, some stocks are known to move inshore and intermingle during spring through autumn. Tagging results indicate that two of the stocks have little or no contact with inshore areas. The Flemish Cap is isolated by a deep trough (Flemish Pass) that forms a geographical barrier and is a deterrent to cod moving across it. Consequently, it is believed that cod inhabiting the Flemish Cap represent an isolated population (Templeman 1962, 1974). Cod occupying the Grand Banks area (divisions 3N and 3O) move east–west and rarely inshore (Templeman 1974). There is also evidence that the components of some migratory stocks remain offshore in summer (Lear et al. 1986).

Biochemical studies have been conducted to determine the relationship between populations of cod from different geographical areas and also spawning stocks. Cross and Payne (1978) suggested, on the basis of electrophoretic studies, the existence of discrete stocks of cod in restricted geographical areas in the Labrador–Newfoundland area. Mork et al. (1985) noted, however, low genetic variation in stocks from the northeast and northwest Atlantic. Smith et al. (1990) also reported low genetic variability between cod from different geographical locations. Using a DNA sequence of a portion of mitochondrial cytochrome *b* gene, Carr and Marshall (1991) noted that the genotypes of cod from Newfoundland differed significantly from those of a Norwegian population. These studies indicate that additional approaches to support or negate the concept of the existence of separate cod stocks are warranted.

The present study was undertaken to ascertain the potential use of protozoan and helminth parasites as biological indicators of stocks of Atlantic cod. Parasites of diverse taxa have often been used to discriminate stocks of marine fishes based on differences in their occurrence and (or) intensity (see MacKenzie 1983; Hemmingsen et al. 1991). Appy and Burt (1982) reported 49 species of metazoan parasites in cod inhabiting the Gulf of St. Lawrence (NAFO division 4T) and the Scotian Shelf (subdivision 4Vn and divisions 4W and 4X) including the Bay of Fundy and Passamaquoddy Bay. Of these, 10 were considered more or less specific for cod or gadids, nine were accidental infections, and the remaining species infected a wide range of marine fishes. Because several of these parasites, both specific and nonspecific, also occur in cod north and east of these previously mentioned areas, we investigated their potential as stock indicators, recognizing that at least five distinct major stocks inhabit the Labrador–Newfoundland area (Templeman 1974). The present study considers cod inhabiting NAFO division 3L to be distinct from those occurring in divisions 2J and 3K, because previous observations (Khan et al. 1980) and the results from tagging experiments (Lear 1984) suggest differences between them.

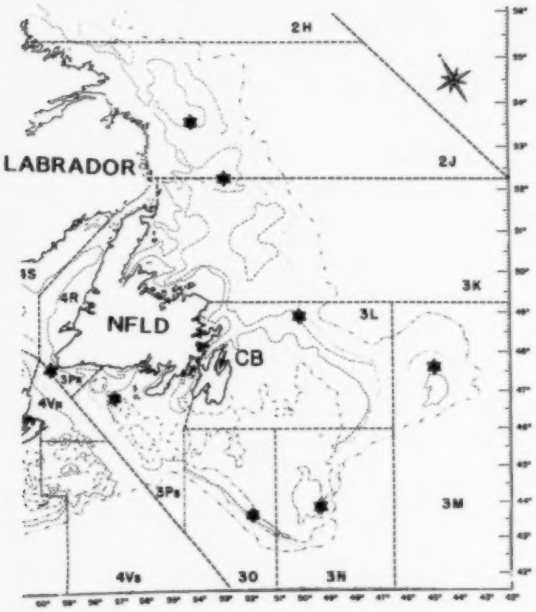
The following criteria were used to assess the potential of parasites as indicators of cod stocks: (*i*) the prevalence and intensity of one or more parasites should be significantly different in fish from the areas compared; (*ii*) the prevalence and abundance of parasitism in all areas should be relatively stable; (*iii*) the parasites' life span should be of long duration, at least one year; (*iv*) the intermediate host(s) should not be dispersed over the entire study area; (*v*) the parasite(s) should not be pathogenic in the length–age classes of the stocks to be studied; and (*vi*) the environmental conditions should be stable and conducive to survival of the parasite(s).

Materials and methods

Cod were captured by otter trawl in the Labrador–Newfoundland area, generally in the locations shown in Fig. 1, by the federal research ships *Gadus Atlantica* and *A.T. Cameron*, during 1981 and 1982, mostly during the prespawning period (Table 1). Approximately 10 fish were sampled from each trawl. Catches occurred at depths of 180–320 m, and bottom temperatures varied from −0.5 to 3.2°C. Samples were deep frozen shortly after capture and were necropsied within 3 months thereafter. The fish examined varied from 47 to 82 cm in total length and the ages, after extrapolation from Fleming (1960) and Pinhorn (1969), ranged from 5 to 12 years in NAFO divisions 2J-3K, 4 to 12 years in division 3L, 4 to 8 years in divisions 3N-30, 4 to 10 years in subdivision 3Ps, and 5–10 years in subdivision 3Pn. An additional sample of cod taken in March 1982 from division 3K was used to compare parasite abundance, primarily that of the acanthocephalan *Echinorhynchus gadi* Zoega in O.F. Müller, 1776, based on length and sex of the fish. The branchial chamber of each fish was also examined for the presence of *Lernaeocera branchialis* (L., 1767). Contents of the digestive tract were recovered following longitudinal dissection and examined for metazoan parasites using routine parasitological procedures (Appy and Burt 1982). Digenetic trematodes were excluded from the study because it was time consuming to identify individual species when large numbers were present. Fillets from 25 fish per sampling area were examined for larval nematodes by candling and, subsequently, by slicing into smaller pieces. A portion of the first gill arch, approximately 20 mm in length, was removed from each of 25 cod taken from each sampling area, fixed in Bouin's fluid, processed by conventional histological methods, and stained with hematoxylin and eosin prior to microscopic examination for cysts of the microsporan parasite *Loma branchialis* (Nemeczek, 1911).

Two additional groups of cod, 47–61 cm in total length (5–8 years old, see Fleming 1960) captured by cod-trap at depths of 10–20 m were examined during the summers of 1982 and 1983 to determine the effect of sex, length, and two parasites, *Trypanosoma murmanensis* Nikitin, 1927 and *Lernaeocera branchialis*, on the abundance of intestinal parasites. *Echinorhynchus gadi* was chosen as an example of the latter, as its prevalence is high (>90%) in cod taken inshore in Conception Bay (47°28′N, 53°01′W; Khan and Kiceniuk 1983). An additional number of cod (404) 46–63 cm in total length were captured by the same gear in the identical location in 1983 and examined to

Fig. 1. Localities (stars) within NAFO divisions/subdivisions from which cod were sampled for parasites. CB, Conception Bay.



determine the prevalence and abundance of *E. gadi* in different length classes. Examination of the intestinal contents followed the procedures outlined previously.

Prevalence and abundance (as defined by Margolis et al. 1982), with standard errors of the mean for the different parasite taxa were calculated after logarithmic transformation (base 10) of the raw data for all groups. Since the distribution of the parasites within the cod samples was not normal, the nonparametric tests (χ^2 approximation) of Kruskal–Wallis (Zar 1974) and Van der Waerden (Hajek 1969) were used. Additionally, the multiple range tests of Waller–Duncan (*K*-ratio *T*-test, see Waller and Duncan 1969) and Welsch (1977) were utilized. Prevalence was compared between groups by the *G*-test (Sokal and Rohlf 1987). The results of these tests, which were similar, are shown as paired comparisons in Table 4 for the Waller–Duncan test.

Results

Several species of parasites observed in Atlantic cod exhibited levels of infection that differed among localities on the continental shelf off coastal Newfoundland and Labrador. The gill protozoan *Loma branchialis* was more prevalent in fish captured in NAFO divisions/subdivisions 2J-3K and 3Pn-4R than in other locations (Table 2). We pooled as a single group the gastrointestinal nematodes, most of which were anisakids. Larval nematodes in fillets were mostly *Pseudoterranova decipiens* (Krabbe, 1878) (Chandra and Khan 1986) and the acanthocephalan observed was *E. gadi*. The copepod *Lernaeocera branchialis* occurred more often and was more abundant on the south and southwestern coasts of Newfoundland (subdivisions 3Ps and 3Pn-4R).

Table 1. Location (NAFO division/subdivision), date, and number of Atlantic cod (47–82 cm in length) sampled off coastal Newfoundland–Labrador.

NAFO location	Area	Date of collection	Number sampled
3M	Flemish Cap	January 1981	118
3Pn-4R	Southern Gulf of St. Lawrence	February 1981	103
2J-3K	Labrador	March 1981	78
3Ps	St. Pierre	March 1981	56
3N-30	Southern Grand Bank	April 1981	79
3L	Northern Grand Bank	April 1981	50
3K	Southern Labrador	March 1982	72
3L	Conception Bay	July 1982	230
3L	Conception Bay	July 1983	404

Statistical comparison by pairs of the samples examined suggested differences in prevalence and abundance (Tables 2 and 3). The prevalence of *Loma branchialis* was significantly different between samples originating from adjacent divisions, i.e., divisions 2J-3K and 3L, 3L and 3N-30, and 3N-30 and 3Ps, but not divisions 3N-30 and 3M and subdivisions 3Ps and 3Pn-4R (Table 2). Similarly, the prevalence of *Lernaeocera branchialis* was significantly greater in division/subdivision 3Ps and 3Pn-4R (which were not different from each other) than in samples taken from the other NAFO divisions. Additionally, the abundance of *E. gadi* was significantly different between samples collected in 2J-3K and 3L, 3L and 3M, 3M and 3N-30, and 3Ps and 3Pn-4R but not between 3N-30 and 3Ps (Table 3). There were also significant differences in groups of cod sampled from adjacent divisions for larval nematodes, i.e., 2J-3K and 3L, 3M and 3N-30, divisions 3L and 3M, and divisions 3N-30 and 3Ps, but not between divisions 3L and 3N-30, and 3Ps and 3Pn-4R. The larval nematode *P. decipiens* was significantly more prevalent and abundant in divisions/subdivisions 3Ps and 3Pn-4R, which also differed from each other, than in the remaining divisions. In essence, paired comparisons revealed that there were more significant differences than similarities between samples taken from adjacent NAFO areas.

Because sex and age or length of the cod used in the present study were unavailable, an examination of a sample captured in division 3K was made to determine the influence of these variables on a parasite's prevalence and abundance, i.e., that of *E. gadi*. No statistical differences in the prevalence or abundance of *E. gadi* were observed between males (91%, $\bar{x} = 23.1 \pm 5.2$, $n = 31$) and females (89%, $\bar{x} = 27.4 \pm 4.9$, $n = 41$). Similarly, the prevalence

Table 2. Prevalence (P) and abundance (A, $\bar{x} \pm$ SE) of some parasites of Atlantic cod (*Gadus morhua*) examined from different areas in the northwestern Atlantic during 1981.

Parasite		NAFO divisions/subdivisions					
		2J–3K (n = 78)	3L (n = 50)	3M (n = 118)	3N–30 (n = 79)	3Ps (n = 56)	3Pn–4R (n = 103)
Loma branchialis	P	90[a]	20[b]	1	2	12[c]	78[a]
	A	—	—	—	—	—	—
Pseudoterranova decipiens in fillets (no./kg)	P	12	8	0	0	24[b]	60[b]
	A	0.2±0.1	0.1±0.0	0	0	0.8±0.1[c]	3.2±0.3[c]
Gastrointestinal nematodes	P	84	85	100	75	96	63
	A	35.6±9.0	9.2±1.6	73.1±12.9	6.5±0.9	23.0±7.2	3.1±0.5
Echinorhynchus gadi	P	95	85	3[b]	60	75	46
	A	29.3±8.1	12.6±2.7	0.8±0.2	6.7±2.0	10.0±2.9	3.0±0.6
Lernaeocera branchialis	P	2	3	0	0	11[a]	12[a]
	A	*[d]	*	0	0	0.1[c,d]	0.2[c,d]

[a]Indicates significant difference ($P < 0.05$) from all groups, but not from each other, based on the G-test.
[b]Indicates significant difference ($P < 0.05$) from all groups, based on the G-test.
[c]Indicates significant difference ($P < 0.05$) from all groups, based on the Waller–Duncan test.
[d]Indicates that the $\bar{x}$ or SE is <0.1.

and abundance in the length classes ≤50 cm (89%, $\bar{x}$ = 26.2 ± 5.8, n = 21), 51–60 cm (87%, $\bar{x}$ = 26.8 ± 6.2, n = 24), and ≥61cm (92%, $\bar{x}$ = 24.0 ± 5.8, n = 17) were not significantly different. Additionally, examination of 404 cod captured inshore revealed that the prevalence and abundance of *E. gadi* in the length classes ≤50 cm (88%; $\bar{x}$ = 9.1 ± 1.2, n = 187), 51–60 cm (87%; $\bar{x}$ = 8.6 ± 1.1, n = 136), and ≥61 cm (90%; $\bar{x}$ = 9.0 ± 1.9, n = 81) were not significantly different.

An additional study was conducted to determine the influence of natural infections of two blood-feeding parasites on the abundance of *E. gadi* in adult (>47 cm in length) cod taken at an inshore location. This study tested the hypothesis that *T. murmanensis* and *Lernaeocera branchialis*, which cause morbidity in cod, could conceivably alter host feeding and ultimately affect the enteric parasites (Khan 1985, 1988). No difference in the prevalence or abundance of *E. gadi* was observed in cod infected with *L. branchialis* (91%, $\bar{x}$ = 9.3 ± 1.2, n = 72), *T. murmanensis* (94%, $\bar{x}$ = 7.4 ± 1.1, n = 62), or without either infection (89%, $\bar{x}$ = 8.1 ± 1.2, n = 96). Moreover, both the prevalence and abundance of *E. gadi* were not significantly different in the samples collected between 1982 and 1983.

Discussion

The parasites that appear to be useful as biological tags in the present study conform to the criteria outlined earlier. Some of the parasites differ significantly in prevalence and (or) abundance in cod inhabiting certain geographical areas. Moreover, with the exception of *P. decipiens*, there is no evidence that either prevalence or abundance has changed over time. The prevalence (95%) of *T. murmanensis* in cod taken in 1985–1986 from division 2J (Lee 1994) was similar to that reported previously (Khan et al. 1980). Neither the prevalence of *Lernaeocera branchialis* nor the prevalence and abundance of *E. gadi* have changed since initial studies were conducted (Templeman and Fleming, 1963; Templeman et al. 1976; Khan and Lacey 1986). However, both the prevalence and abundance of *P. decipiens* have changed in certain areas (McClelland et al. 1985; Chandra and Khan 1986; Brattey et al. 1990) and are associated with an increase of the main definitive hosts, primarily gray (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) (see McClelland 1980; Brattey and Stenson 1993). With the exception of *T. murmanensis*, the life span of the selected parasites approximates or exceeds 1 year. Although the life span of some of the parasites discussed in the present study is relatively short in contrast to their fish host, there is evidence of stability of prevalence and abundance in discrete locations over 2 or more years. MacKenzie (1983) cited a study that reported that a myxozoan infection could persist for years in a fish host. The high prevalence of *T. murmanensis* is believed to be associated with repeated infections of the host and increased longevity of the parasite in cold water. However, little is known of the longevity of the gastrointestinal helminths and the acanthocephalan *E. gadi*. Also, nothing is known of the distribution of intermediate hosts of some of the parasites discussed in the present study. However, the vector of *T. murmanensis* is more abundant in northeastern

than in southwestern locations (Khan 1991). Two of the selected parasites, *T. murmanensis* and *Lernaeocera branchialis*, have been reported to be pathogenic, but mortality occurs mainly in young cod (Khan 1985, 1988). Finally, the environmental conditions in the northwestern Atlantic appear to have been stable during the period that the present study was conducted, but in mid-1980s, a cooling trend was observed, which led to a low in 1991 (Drinkwater 1993). The impact of this trend is unknown, but its occurrence is coincident with a demise of the cod stocks off Labrador–Newfoundland.

The present study, based on parasite data, provides evidence in support of previous observations obtained from tagging and meristic studies and on reproductive and migration patterns that discrete stocks of cod inhabit certain geographical areas of the continental shelf off Newfoundland and Labrador. The prevalence and abundance of one parasite, *E. gadi*, did not appear to be influenced by sex length class, or two pathogenic species, viz., *T. murmanensis* and *Lernaeocera branchialis*. Lee's (1994) investigation of the parasite fauna of cod captured off the Labrador coast (NAFO divisions 2H and 2J) revealed that the abundance of *E. gadi* in various size classes (41–50, 51–60, 61–70, and 71–80 cm total length) was not significantly different. Möller (1976) reported a reduction in total number of intestinal parasites, including *E. gadi*, in four species of marine fishes in captivity. The present study and that of Lee (1994) have shown no changes in the prevalence or abundance of *E. gadi* in Atlantic cod examined in 2 successive years in inshore and offshore locations, respectively. *Echinorhynchus gadi* is potentially the foremost of the metazoan parasitic indicators of cod found in NAFO divisions 2J-3K, as its abundance there was the highest of the areas examined. A current study on the parasite fauna of cod residing along NAFO divisions 2J-3K, has shown that cod taken inshore harbour an abundance of *E. gadi* comparable to fish taken offshore and are thus probably components of the same stock (Lee 1994). Data from tagging studies also support this view (Lear 1984). Two additional studies on two protozoan parasites, *T. murmanensis* and *Myxidium gadi* Georgèvich, 1916, indicate that their prevalences are significantly greater in cod from NAFO divisions 2J-3K than in those from adjacent locations, i.e., division 4R in the Gulf of St. Lawrence and 3L, northern Grand Banks (Khan et al. 1980, 1986). Moreover, a significantly greater prevalence of the gill-inhabiting microsporan *Loma branchialis* in divisions 2J-3K, where 90% of the cod were infected, than in division 3L (prevalence, 20%), adds support to the view that these stocks are distinct. Lear (1984) reported, on the basis of extensive tagging data, that cod inhabiting the northern slope of the Grand Banks (division 3L) represented a stock distinct from the complex of three stocks that occur in 2J and 3K.

The present study provides evidence that only one of the larval nematodes (*P. decipiens*) might be used as a biological tag for cod inhabiting the continental shelf off Labrador–Newfoundland. Larval nematodes, which have a long life span in their piscine hosts, tend to be more useful as biological indicators than adult stages. *Pseudoterranova decipiens* was more prevalent and abundant in cod from divisions 3Pn-4R than in fish from other areas

Table 3. Paired comparisons of the abundance of *Echinorhynchus gadi* and larval ascaridoid nematodes in the digestive tract of Atlantic cod (*Gadus morhua*) from different NAFO areas.

NAFO areas	2J-3K	3L	3M	3N–30	3Ps	3Pn–4R
2J-3K		*	*	*	*	*
3L	*		*	*	*	*
3M	NS	*		*	*	NS
3N–30	*	NS	*		NS	NS
3Ps	NS	*	NS	*		*
3Pn–4R	*	NS	*	NS	NS	

Note: Tests above diagonal line pertain to *E. gadi* and those below are for the larval nematodes.

*, significant differences ($P < 0.05$) between pairs; NS, not significant ($P > 0.05$).

(Templeman et al. 1957; Chandra and Khan 1986; Brattey et al. 1990). The present study also corroborates reports of a substantial increase in the abundance of *P. decipiens* in the fillets of cod originating from subdivision 3Ps (Brattey et al. 1990) since the initial study by Templeman et al. (1957).

There is adequate evidence to indicate that the parasite fauna of a fish species is a reflection of its diet or association with a vector from a given locality (Polyansky 1955; Margolis 1965; Khan 1991). Adult cod are demersal in their behaviour, feeding voraciously after spawning (Templeman 1962). Since the major feeding period of adult cod in the northwestern Atlantic occurs mainly in summer and autumn (Templeman 1962), it is likely that many parasites are acquired during the period that migratory stocks are inshore. Additionally, the prevalence and intensity of *P. decipiens*, a species which uses amphipods, isopods, shrimp, and mysids as intermediate hosts, in cod and other fish species taken in the vicinity of seal colonies indicate acquisition of the infection in inshore locations (see Marcogliese and McClelland 1992). In contrast, the protozoan *T. murmanensis* is acquired more frequently offshore in deep, cold-water locations than in littoral areas where the leech vector *Johanssonia arctica* (Johansson, 1899) is absent (Khan 1991). Nothing is known of the modes of transmission of *Myxidium gadi* and *Loma branchialis*, both of which appear to be useful as biological tags. A study of the food and parasites of juvenile cod from different inshore geographical locations and a comparison with adults, as has been done for pollock, *Pollachius virens*, by Scott (1985), may elucidate the dynamics of host–parasite transmission in the present study area.

Acknowledgements

We are grateful to the Natural Sciences and Engineering Research Council of Canada for financial support, M. Dawe, B. Hines, J. Driscoll, C. Bourgeois, C.V. Chandra, D. Barker, and E. Lee for technical assistance, and the late Mr. R. Wells, Department of Fisheries and Oceans, St. John's, Newfoundland, for providing the opportunity

for this study. I also thank Dr. L.F. Khalil, International Institute of Parasitology, St. Alban's, U.K., for identification of some metazoans, and Drs. G.F. Bennett, D.H. Steele, W. Threlfall, and K. MacKenzie for critically reviewing the manuscript. This is Ocean Sciences Contribution No. 217.

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Parasites as indicators of biological processes, with special reference to sockeye salmon (*Oncorhynchus nerka*)

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Abstract: This paper reviews the applicability of parasites as indicators in studying the biology of their hosts, using examples drawn primarily from the extensive research that has been conducted on the sockeye salmon (*Oncorhynchus nerka*) by scientists of the former Soviet Union and Canada. The data obtained by these researchers has contributed to our knowledge of the feeding and wintering migrations of populations, i.e., local stock complexes, of sockeye and other Pacific salmon. In some local stocks of sockeye salmon, millions of fish carry such biological tags as specific parasites and scale marks that facilitate the study of their seasonal migrations. Parasite indicators are also useful in distinguishing fish of various subpopulations and trophic groups within populations of freshwater species, while an analysis of the parasite fauna of juvenile fish may reveal those fish hosts that inhabited the river in the recent geological past but are now locally extinct. In regulating subpopulations of sockeye salmon, parasites are the effect rather than the cause of mortality. The above examples are evidence of the successful application of parasites for studying biological processes.

Résumé : Nous examinons la possibilité d'employer les parasites comme indicateurs pour étudier la biologie de leurs hôtes, en prenant surtout des exemples dans les recherches approfondies menées sur le saumon rouge (*Oncorhynchus nerka*) par des scientifiques de l'ancienne URSS et du Canada. Les données recueillies par ces chercheurs ont enrichi nos connaissances sur les migrations d'alimentation et d'hivernage des populations, c'est-à-dire sur les complexes localisés de stocks de saumons rouges et d'autres saumons pacifiques. Dans certains stocks localisés, des millions de poissons portent des étiquettes biologiques constituées par des parasites spécifiques et des marques sur les écailles, qui facilitent l'étude de leurs migrations saisonnières. Les parasites indicateurs sont aussi utiles pour distinguer les poissons de diverses sous-populations et de groupes trophiques au sein des populations dulcicoles, tandis qu'une analyse de la faune parasite des poissons juvéniles peut révéler quels hôtes habitaient le cours d'eau dans un passé géologique récent mais ont maintenant disparu localement. Dans la régulation des sous-populations de saumon rouge, les parasites sont l'effet plutôt que la cause de la mortalité. Les exemples ci-dessus confirment l'utilité des parasites pour étudier les processus biologiques.

[Traduit par la Rédaction]

The use of parasites for studying the ecology of fishes has a long history. Through studies dealing with migratory fishes, the spatial structure of populations of different species, intraspecific ecological forms or sympatric species, zoogeography, and population dynamics, parasites have been successfully used to obtain more detailed knowledge about the biology of a wide variety of marine, diadromous, and freshwater fishes. This review is an attempt to analyze the results of the above-mentioned studies in using parasites as indicators to provide information on the biology of their host fishes, with emphasis being given to Pacific salmon (*Oncorhynchus* spp.).

Received April 5, 1994. Accepted December 7, 1994
J12674

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Parasites as indicators of spawning and nursery areas

Identification of spawning and nursery areas of fish has always been of great interest. However, traditional methods of identification, e.g., tagging, have restrictions that do not allow sufficient information about the biology of a given species to be obtained. Therefore, researchers have paid much attention to the use of parasites as indicators of the geographical origin of fish.

In this context, Pacific salmon provide an illustrative and helpful example. Not only zoologists but specialists in related disciplines have concentrated on species of this

genus, because these fishes are capable of making extensive feeding migrations to offshore areas and return to spawn only in those rivers or lakes where their parents spawned (Hasler 1966; Foerster 1968; Groot and Margolis 1991).

Sockeye salmon (*Oncorhynchus nerka*) is an ideal model species owing to characteristics such as its remarkable duration of freshwater life, 1 year in rivers and 1–3 years in lakes. When living in fresh water, young sockeye can be infected by a variety of parasites before reaching the smolting stage. Some of the parasites survive throughout the marine life of the fish and can be found in adult sockeye on the spawning grounds (Margolis 1982). Cases have been reported where the prevalence of infection reached almost 100% in populations of millions of these fish (Konovalov 1975).

The offshore fishery for Pacific salmon, which began in the early 1950s, raised a number of questions. (*i*) Do American and Asian salmon intermix in the North Pacific? (*ii*) Are American and Asian stocks caught mainly by the Japanese fishery? (*iii*) What recommendations should be given and implemented to ensure a sustainable fishery? These and other questions were addressed by the scientists of the North Pacific countries.

Studies performed on many species of fish (e.g., Dogiel and Bykhovsky 1939; Shulman 1954; Bishop and Margolis 1955; Kabata 1957, 1959; Sindermann 1957*a*, 1957*b*) have shown that parasites can be reliable indicators of host geographical origin. Beginning in 1955, studies on sockeye salmon were initiated in Canada by Margolis (Margolis 1963, 1965; Margolis et al. 1966). In Russia, Mamaev and colleagues began studying the parasites of the Far Eastern Salmonidae in 1957 (Mamaev et al. 1959). The author of this review worked in Mamaev's group on Kamchatka while a student and later continued his own independent research.

Margolis (1963) proposed the use of two parasites, *Triaenophorus crassus* Forel, 1880 and *Truttaedacnitis truttae* (Fabricius, 1794) as indicators for sockeye salmon of American and Asian origin, respectively. I have designed a combined method for identifying the origin of sockeye salmon using not only their parasites but also peculiar scale marks that differ between fish examined during the freshwater stage of their life cycle and during the first months of marine life (Konovalov 1975). For example, Bristol Bay sockeye salmon can be easily distinguished from all other geographical populations by possessing a very wide zone in the scale mark indicating growth during their first marine summer. This combined method allows us to determine the geographical origin of sockeye salmon taken in offshore waters of the North Pacific Ocean.

The traditional method of tagging with plastic tags is more obvious, in that anyone who catches a tagged fish can easily trace its migration route. Unfortunately, this method is much more expensive and labour intensive than the use of parasite indicators or the combined method. In addition, conventional tagging does not tag millions of fish, as the parasitological or combined methods do, because the indicator parasites infect the majority of the fish population and the peculiar scale marks are found in each fish. This contributes remarkably to our knowledge about the ocean life of sockeye salmon.

French et al. (1976) published the results of a comprehensive survey in which the combined method was used by fisheries biologists to study the distribution of sockeye salmon in offshore waters. Since then, data about the presumed origin of sockeye salmon caught offshore have been obtained using various techniques and integrated with information on migration routes and areas of distribution of the fish during its ocean life (Forrester 1987; Burgner 1991; Margolis 1991).

Dogiel and Bykhovsky (1939) were the first Soviet researchers to explore theoretically the suitability of parasites as indicators of origin for different species of sturgeon. Later, Shulman et al. (1959) described the possibilities for identifying fish from different spawning grounds in lakes. Lüling (1951), Perlmutter (1953), Templeman (1953), Sindermann (1961*a*, 1961*b*), Yanulov (1962), and a number of other authors worked on the problem of revealing the locality of origin of redfish (*Sebastes marinus* and *S. mentella*) by using parasite indicators. Similar studies were conducted on herring (Bishop and Margolis 1955; Sindermann 1957*a*, 1957*b*, 1961*a*) and on whiting, cod, and haddock (Shulman 1950; Kabata 1957, 1959, 1963, 1967; Sherman and Wise 1961; Wise 1963).

Research performed worldwide on different fish species during the second half of this century has confirmed the ideas put forth by Margolis (1965). Parasites of fish can provide valuable evidence about the biology of their hosts, including the geographical origin of migratory species (MacKenzie 1983, 1985; Lester 1990; Williams et al. 1992; Arthur and Albert 1993).

Discrimination of local stocks

Modern population biology distinguishes two main levels in the spatial structure of species: the subpopulation level (local populations, subpopulations, subisolates, demes) and the population level (populations, isolates, local stocks) (Kimura and Ohta 1971; Roughgarden 1979; Krebs 1985). These two levels differ in values of coefficient of migration (m) (Li 1955), which is within the range 10^{-1}–10^{-3} at the subpopulation level and is about 10^{-5} at the population level. A good example of the subpopulation level is sockeye salmon that spawn in springs, streams, rivers, and littoral areas of lakes (Hartman and Raleigh 1964; Konovalov 1983).

While Margolis developed the method for differentiating geographical populations, I sought to make this method applicable to the population level (Konovalov 1975). Special attention was given to distinguishing sockeye salmon of the principle lake populations of Kamchatka in the ocean. I found that only two types of cyst of plerocercoids of the cestode *Diphyllobothrium* sp. and the marks characteristic of the freshwater and estuarine zones on the scales of sockeye were needed to clearly distinguish fish of Kuril Lake from those originating from Asia and North America.

The local stock of Kuril Lake has the greatest number of spawners compared with other Kamchatkan stocks and fish from this population are most frequently found in offshore catches. Birman and Konovalov (1968) and Konovalov (1975) examined the distribution of Kuril Lake sockeye in the coastal and offshore waters during their first weeks

and their spawning migration to the Ozernaya River. Thirty years of research permits me to conclude that hardly a more efficient and inexpensive method for discriminating between fish of different local stocks can be offered.

It was observed from year to year that, in the largest population of sockeye salmon on Kamchatka, each smolt that migrates to the ocean is infected by plerocercoids of *Diphyllobothrium* sp. The scale marks present in each smolt permitted all fish of the Kuril Lake local stock which were caught in coastal and offshore waters to be identified, eliminating the necessity of catching large numbers of fish for research. Initially, there were few opportunities to study the offshore distribution of sockeye salmon belonging to the Kuril Lake local stock. However, considerable data have been obtained on their distribution from the first weeks until they migrate to the Ozernaya River (Birman and Konovalov 1968; Konovalov 1975). Recently, very interesting studies on the migration routes of sockeye salmon of different local stocks during the first month of marine life have been published (Bailey et al. 1988*a*, 1988*b*; Groot et al. 1989; Garnic and Margolis 1990).

Many of the above parasitological papers deal with the population level, but none estimate the coefficient of migration (Konovalov 1983) and therefore, they fail to accurately determine whether the populations studied belong to geographical populations, local stocks, or subpopulations. However, their spatial structure on the fishing grounds and the status of the populations are highly important with regard to the commercial fishery. In this regard there are two main scenarios: (*i*) complete mixing of several populations in the fishing area and (*ii*) complete separation of several populations in the fishing area. For sustainable fisheries, if several fish populations mix it is necessary to differentiate fishing pressure on individual populations and regulate the catch for each individual population (Konovalov et al. 1975). This scenario applies to Pacific salmon in the North Pacific Ocean, the Bering and the Japan seas, and the Sea of Okhotsk.

The theoretical aspect of the spatial structure of different populations (local stocks) includes estimation of the extent of feeding and wintering areas. To determine this, it is sufficient to identify a small percentage of the fish in each local stock using parasite indicators. If the feeding and wintering areas combine, no competition should be found between the fish belonging to different local stocks, as has been described for Pacific salmon (Konovalov 1975).

Analysis of the parasite fauna acquired via feeding provides a more detailed means to estimate the rate of similarity in food and prey preferences than do other methods. Margolis (1965) and other researchers (Konovalov 1975; Kuperman 1975, 1978) reported that parasites that use intermediate hosts can characterize the specific diet for a year and more. No other method is known for assessing food similarity in fish of different populations over a considerable time span.

Data on the mixing of local stocks of sockeye salmon and other species of Pacific salmon in the offshore feeding areas obtained by various methods has shown the absence of any feeding competition (LeBrasseur 1972; Konovalov 1975; Karpenko 1979; Healey 1980; Peterman 1980). This conclusion agrees with the data obtained from the culture and ranching of Pacific salmon. In particular, chum salmon (*Oncorhynchus keta*) of Hokkaido and Honshu Islands that migrate to the North Pacific Ocean for feeding show a high survival rate until adulthood (Kobayashi 1980). In recent years, artificial propagation of chum salmon in Japan has contributed as much as 50 thousand tons and returns of adult fish, which are in conformity with the above conclusion.

Fish parasites as ecological indicators at the subpopulation level

Studies of "homing" of sockeye salmon spawners to various creeks, springs, and streams of the same lake system that involved tagging and transferring of fish from their native spawning grounds (Hartman and Raleigh 1964) made a deep impression on me. These studies, together with physiological work (Hasler 1966), became the basis of my long-term studies of the subpopulation level. It had been supposed that a local stock of sockeye salmon in any lake would subdivide into self-regulated units known as subpopulations (subisolates). For about 25 years me and my colleagues studied this population level in animals, and parasites were an important source of knowledge about subpopulation structure and function (Konovalov 1980). The first part of the above study involved using parasites to differentiate young sockeye of various subpopulations. Larvae of sockeye salmon spend from several days to several weeks on the spawning grounds but do not become infected during this period. Therefore, in this case the parasite-indicator method cannot be applied. It is noteworthy that other data suggest the possibility of differentiating some freshwater fish subpopulations (Shulman et al. 1959). This is remarkable, since the number of subpopulations in lake populations of freshwater fishes is usually not large.

Studies on the parasite fauna of young sockeye have shown that yearlings and smolts with different types of scale marks also differ in the prevalence and intensity of infection with freshwater parasites. Thus, in yearlings of spring sockeye salmon with typical summer sclerites and distinct winter check (type *b*) and yearlings with distinct summer sclerites but without winter sclerites (type *d*) (Clutter and Whitesel 1956) reduced prevalence and intensity of infection with plerocercoids of *Diphyllobothrium* sp. were found (Konovalov et al. 1970; Konovalov 1980). The yearlings with two false checks (type *f*:- false two-yearling) or with one false check (type *h*) (Clutter and Whitesel 1956; Mosher 1969; Konovalov 1980) were often found among spring sockeye migrating to the ocean at the age of 1 year. These types of scales are typical in summer sockeye of the second year of freshwater life.

Fish with *h* and *f* scale types showed higher prevalence and intensity of infection with plerocercoids of *Diphyllobothrium* sp. (Table 1). The first intermediate hosts of this cestode are *Cyclops strenuus* (Guttowa 1963) and some species of the genus *Diaptomus*. Relative abundance in the yearlings attributed to types *b* and *d* was 0.57 and 0.41,

Table 1. Parasite-indicators of trophic divergence among yearling sockeye salmon from Azabachye Lake with different scale marks (*b*, *d*, *f*, and *h*).

Species of parasite	*b* prevalence (%)	Intensity (n = 147)	*d* prevalence (%)	Intensity (n = 53)	*f* prevalence (%)	Intensity (n = 53)	*h* prevalence (%)	Intensity (n = 94)
Eubothrium salvelini	9.5	2.5 (1–11)	15	1.8 (1–3)	—	—	2.1	2 (1–3)
Diphyllobothrium sp. plerocercoid	37.4	1.53 (1–6)	34	1.2 (1-3)	96	4.6 (1–10)	94.7	4.5 (1–10)
Cyathocephalus truncatus	2.7	1.0 (1)	5.7	11.3 (1)	11.3	11.3 (1–32)	—	—

Note: Values are means followed by ranges in parentheses.

respectively, while in those of types *f* and *h*, 4.83 and 4.26, respectively.

Although being of low prevalence and intensity of infection, two other parasite-indicators were also of interest. Yearlings with scales of type *f* were not infected with the cestode *Eubothrium salvelini* Schrank, 1790, those with scales of type *h* were rarely infected, and those with scales of types *b* and *d* showed a higher abundance of this parasite. This proves that *Cyclops scutifer* (Boyce 1974; Kuperman 1978; Margolis 1982) is a common prey for the latter groups of yearlings, while yearlings with scales of type *h* rarely use it as a food item.

Yearlings with *f* type scales are infected with the cestode *Cyathocephalus truncatus* (Pallas, 1781) but those with *h* type scales are uninfected. Smolts become infected with this parasite when feeding on benthic crustaceans of the family Gammaridae (Gynezinskaya 1958). This parasite was never found in yearlings from pelagic areas of lakes. Smolts with *f* type scales either live in the littoral zone for a short time or occasionally feed there. In this case, the young sockeye are capable of feeding both on benthic organisms in the littoral zone and on zooplankton in pelagic waters.

Sockeye salmon retain plerocercoids of *Diphyllobothrium* sp. throughout their entire life. This specific feature facilitated study of the occurrence of this parasite in spawners with different scale types in 20 subpopulations of sockeye of Azabachye Lake. Some results of this 20 year study were reported earlier by Konovalov (1980). This work and other data confirm the existence at the subpopulation level of trophic groups of young sockeye that prefer to feed on a wide variety of organisms rather than on a specific food item.

Examination of the prevalence and intensity of infection of plerocercoids of *Diphyllobothrium* sp. in male and female sockeye salmon with identical scale marks has shown marked sex-related differences within subpopulations. For comparative study, 284 samples were taken from the subpopulations of the Azabachye Lake population. Males, as a rule, were characterized by a higher level of infection and, hence, by more active consumption of copepods, than were females. The average intensity of infection with *Diphyllobothrium* sp. differed by as much 14 times between male and female fish from a given subpopulation (Konovalov and Butorina 1984).

A similar study has been conducted on the subpopulations of sockeye salmon of Iliamna Lake, Kvichak River system, Alaska. It was found that infections in males were three times greater than in females, because the former consumed three times more planktonic organisms (*Cyclops* spp., *Diaptomus* spp.) (Konovalov 1980). The fact that males and females in subpopulations of sockeye salmon from Asian and American populations differ in feeding preference testifies to the wide-spread character of this phenomenon. Thus parasite indicators are a helpful means to distinguish trophic specialization and to apply the niche theory at the subpopulation level.

The niche theory, which was advanced in the early 1900s, considers intra- and inter-specific competition and its relation to food and competition (Elton 1934). As long as investigations at the intraspecific level require the discernment of individuals of various groups, the niche theory has focused on interspecific relationships (Whittaker and Levin 1975). In this regard, studies of species of the same genus or sympatric species are a very promising means to promote a better understanding of trophic divergence (see Gause 1934; Hardin 1960).

In fish species of problematic taxonomic status, food preferences differ considerably in the salmon lakes of the Kamchatka Peninsula and North America. For example, there are two easily distinguished forms of freshwater char (*Salvelinus alpinus*). The predatory char is pelagic, inhabiting the limnetic waters of lakes, while the benthophagic form prefers littoral and bottom areas. Though morphologically similar (Savvaitova 1973), these fish can be easily distinguished by their stomach contents. One group feeds only on small fishes (*Gasterosteus aculeatus*, *Pungitius pungitius*, young Pacific salmon), while the other feeds exclusively on gastropod molluscs.

The use of parasite indicators allows us to discriminate exactly between these two forms of char (Konovalov 1975). *Eubothrium salvelini* and plerocercoids of *Diphyllobothrium* sp. are common in the predatory form, while the trematode *Phyllodistomum conostomum* (Olsson, 1876) is found in the benthophagic form (Table 2). In Dalneye Lake these parasite-indicators occur mostly in the pelagic

Table 2. The parasite indicators of limnetic (piscivorous) and benthic (benthophagic) forms of Arctic char in Azabachye Lake.

	Limnetic		Benthic	
Parasite	Prevalence (%)	Intensity	Prevalence (%)	Intensity
Eubothrium salvelini	87	112 (14–372)	13	23.5 (23–24)
Diphyllobothrium sp. plerocercoid	100	48 (13–98)	—	—
Phyllodistomum conostomum	—	—	67	200 (30–1160)

Note: Values are means followed by ranges in parentheses.

environment and hence in the predatory (limnetic) form of this fish. In benthophagic fish, *Diphyllobothrium* sp. and *Diplostomum* sp. are absent (Table 3). Thus sympatric species or forms that display specific ecology and food preferences differ markedly in their parasite faunas, and in the prevalences and intensities of infection.

Bentzen and McPhail (1984), Schluter and McPhail (1992), McPhail (1993), and Schluter (1993) described the sympatric species of the genus *Gasterosteus* in salmon lakes as limnetic and benthic forms. Although there was no comparative study of the parasite faunas of these species, parasites could certainly be applied to distinguish their ecology. There is no doubt that trophic specialization is common in species of the same genus, in intraspecific ecological forms, and in subpopulations.

It is known that males and females can differ in diet. Schoener (1974) described this phenomenon for island lizards and Storer (1966) for North American hawks. These investigations have shown that difference in body size between males and females is the main factor responsible for trophic divergence. Partridge and Green (1985) analyzed the intraspecific feeding specialization of various animal groups based on sex and age differences. Subsequently, many other authors have confirmed that males and females differ in their feeding patterns. This fact may account for the intraspecific trophic divergence. The optimal foraging theory explains trophic divergence at this subpopulation level as a means to avoid feeding competition between males and females. In addition, sex-related trophic specialization at the critical period when larvae are incapable of actively searching for prey is a very important ecological adaptation. This adaptation is realized through the maximum utilization of food by each larva in these trophic groups, owing to enzyme specialization and the principle of maximally efficient exploitation of different food resources.

I believe that the parasite fauna and parasite indicators may provide useful information about the intraspecific ecology of animals.

Parasite indicators and extinct ichthyofauna

In studying the parasite fauna of juvenile and adult northern pike (*Esox lucius*) in the Penzhina River (Chukotka), I have found only nine species of parasites in adult pike but 22 in the juveniles. The number found in adult pike is surprisingly small compared with that reported from other rivers within the circumpolar area (Konovalov 1975). Among the parasites found in juvenile pike, only *Triaenophorus nodulosus* (Pallas, 1781) and *Diplostomum spathaceum* (Rudolphi, 1819) have intermediate hosts, while five other species occur in the juvenile and adult pike of other rivers.

Paradoxically, all other parasite species displayed high specificity for certain species of Cyprinidae and Percidae, and have not been previously reported from pike. The first group includes eight species specific to Eurasian minnow (*Phoxinus phoxinus*) which are infrequently found in other cyprinids. *Phoxinus phoxinus* is rare in the Penzhina River and it was impossible to obtain a sufficient sample size for parasitological study.

The above observations suggest that the parasites *Paratrichodina incisa* Lom, 1959; *Tripartiella lata* Lom 1963; *Trichodina tumefaciens* Davis, 1947; *T. nemachili* Lom, 1970; *T. intermedia* Lom, 1960; *Myxidium macrocapsulare* Auerbach, 1910; *Dactylogyrus borealis* Nybelin, 1936; and *Gyrodactylus phoxine* Malmberg, 1956 use juvenile *Esox lucius* as a substitute for *P. phoxinus*. Only five of these parasite species were found in other rivers of northeast Asia (Pugachev 1984). With the occurrence of *P. phoxinus* in the Penzhina River, it is probable that parasites specific to this fish have secondarily infected juvenile pike.

A second group specific to fishes of the families Cyprinidae and Percidae consists of seven parasite species: *Sphaerospora minuta* Konovalov, 1967; *Myxobolus dujardini* (Thélohan, 1899); *M. aliensis* Konovalov, 1967; *M. musculi* Keysselitz, 1908; *Trichodina nigra* Lom, 1961; *Gyrodactylus cernuae* Malmberg, 1956; and *G. decorus* Malmberg, 1956. Pugachev (1984) found some of these parasites in cyprinid fishes of East Siberia. For *Myxobolus dujardini*, the hosts are *Rutilus rutilus*, *Leuciscus idus*, and *L. leuciscus* in the basin of the Ob River and *Carassius auratus gibelio* in the basin of the Lena River. *Trichodina nigra* was reported for *R. rutilus*, *L. idus*, *L. leuciscus*, and *C. auratus gibelio* in the basin of the Ob River. *Gyrodactylus decorus* is specific to *Scardinius erythrophthalmus*, *Alburnus alburnus*, and *R. rutilus*, and *G. cernuae* to *Acerina cernuae* and *Perca fluviatilis* (family

Table 3. The parasite indicators of limnetic (piscivorous) and benthic (benthophagic) forms of Arctic char in Dalneye Lake.

	Limnetic		Benthic	
Parasite	Prevalence (%)	Intensity	Prevalence (%)	Intensity
Eubothrium salvelini	95	22 (5–67)	32	9 (1–29)
Diphyllobothrium sp. plerocercoid	70	6 (1–25)	—	—
Protepcephalus exuguus	100	166 (8–615)	42	22 (2–15)
Diplostomum spathaceum	100	24 (10–37)	—	—

Note: Values are means followed by ranges in parentheses.

Percidae). Two species of Myxosporida (*Sphaerospora minuta* and *Myxobolus aliensis*) were found only in juvenile pike in the Penzhina River, indicating that these parasites probably secondarily infected juvenile pike from host species that are now locally extinct.

This phenomenon can be explained by the previous occurrence of the same species of cyprinids and percids in the Penzhina River (Lindberg 1955; Svetovidov and Dorofeeva 1963). Palaeontological data have confirmed that *A. alburnus* and *S. erythrophthalmus* occurred in East Siberia but vanished in the late Pliocene and early Pleistocene (Tsepkin 1967). It is well recognized that many groups of animals are very similar in morphology and biochemistry during the early stages of their ontogeny. This fact provides additional support for the hypothesis that species-specific parasites of cyprinid and percid fishes have survived in the Penzhina River by switching from their traditional hosts to juvenile pike.

The Chukotka region experienced changes in climate and sea level in the Cenozoic (Lindberg 1955; Hopkins 1967, 1976). These impacts could be responsible for the extinction of some fish species that occurred in downstream rivers. The species-specific parasite indicators of locally extinct cyprinid and percid fishes have survived on juvenile pike. This also suggests that some of these specific hosts survived in the rivers of western and eastern Siberia (Pugachev 1984) and that they were present as recently as the Pliocene and Pleistocene periods. Apparently, some parasites without intermediate hosts are capable of surviving during geological perturbations. Their presence furnishes additional information about the recent geological past of the river basins.

Parasites and their population dynamics in host populations

Elton (1942) conducted pioneering research on this problem and was the first to emphasize the importance of parasites in regulating host numbers. He used statistical data to study relationships between parasites and hosts, especially during epizootics. Elton concluded that in some regions almost all voles died because of epizootics, and that epizootics never occurred in the same region during successive years.

Anderson (1979) examined the epidemic concept and concluded that host–parasite associations exist only under stable co-existence. When co-existence is disturbed, epidemics break out. This conclusion raises two questions. (*i*) Can an epidemic take place independent of population numbers? (*ii*) What would be the cause of the epidemic? A century of studies on Pacific salmon can provide answers to some of these questions, thanks to the extensive knowledge accumulated about natural and artificial populations of this fish and their unusual biological properties.

Not a single paper has been published about epizootics in natural populations of Pacific salmon. The intricate character of the host–parasite relationship is amazing. For example, cases have been reported where *Myxobolus neurobius* Schuberg and Schroder, 1905 was found in the brain in almost 100% of natural populations of sockeye salmon (Konovalov 1975; Quinn et al. 1987). However, this parasite appears to have no effect on the normal physiological state of infected fish and infected sockeye rarely fail to find their native spawning grounds (Konovalov 1983).

Among the other species, the myxosporidan *Henneguya zschokkei* (Gurley, 1894) is a common parasite of several salmonid species that affects most severely the musculature of adult coho salmon (*Oncorhynchus kisutch*) (Konovalov 1975). It would be natural to assume that high pre-spawning mortality occurs in infected coho populations, but this is not the case. The "cysts" begin to develop rapidly in adult fish when they return to coastal areas to spend the last weeks of life. After the post-spawning death and decomposition of the fish, the spores spread rapidly in the streams and rivers.

Margolis (1982) reported a shortage of data on the impact of parasites on the health of fish in nature and gave examples typically faced in fish farming. Smith (1973) discussed the reduced growth rate and swimming performance observed in young sockeye infected by the cestode *Eubothrium salvelini*. Estimation of mortality rates resulting from parasitic infections in wild populations is a difficult task and requires accurate information on parent–progeny relationships at the subpopulation level or the application

of nontraditional methods. Pathogenesis owing to parasites may occur provided that the parasite–host relationship is very short or where the intermediate host is weakened to the extent that it becomes easy prey for the definite host. Examples of this phenomenon are cestodes of the family Ligulidae, whose plerocercoids, upon infecting the fish intermediate host, grow rapidly and reach considerable size. Infected fish swim slowly and mainly at the surface, and as a result, are easily caught by birds.

Epizootics in Pacific salmon are known only in fish farming, owing largely to the high stocking densities used. Anderson (1979) suggested that the increased density of animals in an artificial population would lead to enhanced pathogenic effects of parasites. The high population densities used in fish farms may cause stress. A good example is the myxosporidan *Myxobolus cerebralis* (Hofer, 1903). In nature this parasite occurs less frequently than *M. neurobius*, however, in commercial fish breeding operations it may cause high losses (Margolis 1982).

The long-term research conducted at the subpopulation level on 20 subisolates of spring and summer sockeye salmon in Azabachye Lake has been already mentioned. Data have been obtained from about 300 cases which demonstrate the relationship between parent–progeny numbers with regard to epizootic situations (Ricker 1954; Konovalov 1989, 1990). Population density in sockeye spawners during spawning was found to be the only factor that dramatically affected the numbers of progeny. Optimum density of spawners on the spawning grounds predetermines maximum numbers of adult fish in the first progeny (F_1) (Konovalov 1989, 1990). There has not been a single parasite-induced epizootic, including diseases common in salmon rearing, observed in sockeye salmon on the spawning grounds (Margolis 1982).

In population density-dependent relationships, the greater the density of spawners, the more aggressive their behaviour. The efficiency of reproduction dramatically declines because of the fierce fights between males, females, and males and females. Such behaviour results in heavy losses of eggs owing to males and females spawning outside the redds and a large number of dead females with eggs partially or completely retained in the body cavity. Comprehensive statistics have been collected on the efficiency of reproduction based on the examination of post-spawning male and female gonads under conditions of high spawner density. These studies confirmed that under such conditions males and females did not reproduce and their milt and eggs remained within the body cavity. Thus, high spawner density leads to low reproductive efficiency.

An interesting observation has been made during the examination of the embryogeny of fertilized eggs following the deliberate enhancement of prespawning concentrations of males and females on the spawning grounds. In these embryos, the mortality rate was 90–95% compared with the control. Chebanov (1994) has confirmed these results in a number of experiments with sockeye salmon of the Bolshaya River, Kamchatka. These long-term studies concluded that population density-dependent relationships in salmon on the spawning grounds regulate the fish numbers in the next generation. Thus, parasite-induced epizootics are the result, rather than the cause, of population stress and reduced resistance to disease.

This hypothesis offers a reasonable explanation for the high frequency of epizootics in fish breeding facilities and the difficulty in detecting their occurrence in natural populations. It also contributes to a better understanding of exceptions to current ideas about parasite–host co-existence. As Anderson (1979) concluded, "theory certainly supports the widely held opinion that epidemics are essentially density-dependent phenomena."

Discussion

For more than 30 years I collected data on the value of parasite indicators as a source of biological information. Parasites are now widely used to determine the origin of fish populations and the spatial structure of species (Margolis 1982; Gaevskaya 1989). By applying different methods of tagging and through understanding the geographical distributions of populations and the migrations of fish, it became possible to determine the foraging and wintering areas of large population complexes, such as those of Bristol Bay, the Gulf of Alaska, and the Kamchatka Peninsula (French et al. 1976).

The high prevalence of some parasites has allowed their analysis to provide insight into the spatial structure of different local stocks (Konovalov 1975). Additionally, from an economic standpoint, the use of parasites as indicators is much cheaper than other methods of tagging.

Parasites that require intermediate hosts in their life cycles are useful indicators of host feeding habitats. Some parasites are known to live in their definite hosts for a year or more, sometimes until the death of the host. Thus parasite-indicators may be useful in estimating the number of prey (intermediate hosts) eaten by the fish over several months to several years. No other method allows such estimation in natural populations and hence, the ability to distinguish trophic groups within subpopulations.

The study of parasites can provide additional knowledge about the history and origin of the ichthyofauna of geographical units (Polyansky 1958; Shulman 1958; Margolis 1982; Pugachev 1984; Gaevskaya 1989). Parasites that are capable of surviving despite the extinction of their hosts may shed light on the ichthyofauna of the past. As mentioned above, parasites of cyprinid and percid fishes have survived and persist in the basin of the Penzhina River, providing evidence that these fish families occurred there in the past.

Krebs (1985) regarded parasites as an essential factor ensuring regulation of animal populations, and Lack (1954) discussed the role of parasites in the regulation of bird populations. Andrewartha and Birch (1984) used the case of *Myxoma* virus in Australian rabbits as an example of population control by parasites. This example agrees with the hypothesis of Anderson (1979) and with my own views on the causes of epizootics. The *Myxoma* virus that infected rabbits in Australia is an obvious example of the beginning of a parasite–host relationship. This virus originated in South America, while the rabbit is of European origin. Their relationship began to develop in Australia in the

middle of this century. Thus, the case of *Myxoma*-induced epizootics illustrates a persistent parasite–host relationship.

In animals, stress can increase owing to different natural and artificial stressors, leading to a sharp decrease in resistance. This can lead to a growing number of weakened animals and an epizootic situation. High mortality from parasitic infection in natural populations can result from either a high density of animals or from environmental catastrophes. Parasitological studies have shown that parasites, as a rule, are not the cause of drastic fluctuations in natural animal populations.

Parasitology is a science that offers great potential as a source for gaining knowledge on host biology. It is regrettable that this applicability has not yet been fully recognized. A possible explanation of this may lie in the complexity of interdisciplinary synthesis. Still, for economic reasons, the use of parasites appears to be a practical idea.

Acknowledgements

Since my very first steps in science I have been supported and encouraged by S.S. Shulman, Yu. I. Polyansky, L. Margolis, Z. Kabata, and Yu.L. Mamaev. In particular, I have greatly appreciated the aura of understanding and friendly concern that was radiated by L. Margolis and S.S. Shulman. It was my good fortune to meet such mentors, and with age I have tried to be a similarly good patron to my younger colleagues.

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Parasites of the hakes *Merluccius australis* and *M. hubbsi* in the waters around the Falkland Islands, southern Chile, and Argentina, with an assessment of their potential value as biological tags

K. MacKenzie and M. Longshaw

Abstract: Samples of *Merluccius australis* (113 fish) and of *M. hubbsi* (195 fish) were collected from 16 positions on the Falklands and Argentine shelves and 1 off southern Chile. Twenty-eight fungal, protozoan, and metazoan parasite taxa, of which 19 were identified to the species level, and cysts of unknown etiology, were found. These included one new species of myxosporean protozoan found in both host species, three species that are new to both species of hake, three species not previously reported from *M. australis*, and one not previously reported from *M. hubbsi*. The most promising biological tags for stock identification, where long life spans are important, are an unidentified fungus; the protozoans *Alatospora merluccii* Kalavati, Longshaw and MacKenzie, 1995, *Goussia* sp., *Kudoa rosenbuschi* (Gelormini, 1944), *Microsporidium ovoideum* (Thélohan, 1895), *Myxidium baueri* Kovaleva and Gaevskaya, 1982, and *Myxoproteus meridianalis* Evdokimova, 1977; the metacestodes *Grillotia* sp. and *Hepatoxylon trichiuri* (Holten, 1802); and the parasitic copepod *Trifur tortuosus* Wilson, 1917. The adult digenean *Elytrophalloides oatesi* (Leiper and Atkinson, 1914) showed some potential as a tag for following seasonal migrations of hake.

Résumé : Nous avons prélevé des échantillons de *Merluccius australis* (113 poissons) et *M. hubbsi* (195 poissons) à 16 endroits sur le plateau continental des Malouines et de l'Argentine, et à un endroit au large du sud du Chili. Nous avons trouvé 28 taxons parasites, champignons, protozoaires et métazoaires, dont 19 ont été identifiés jusqu'au niveau spécifique, ainsi que des kystes d'étiologie inconnue. Il s'agissait notamment d'une nouvelle espèce de protozoaire myxosporidien trouvée chez les deux hôtes, de trois espèces qui sont nouvelles chez ces deux merlus, de trois espèces qui n'avaient pas été signalées jusqu-là chez *M. australis*, et d'une espèce qui n'avait pas été signalée chez *M. hubbsi*. Les marques biologiques les plus prometteuses pour l'identification des stocks, lorsque la longévité est importante, sont : un champignon non identifié; les protozoaires *Alatospora merluccii* Kalavati, Longshaw et MacKenzie, 1995, *Goussia* sp., *Kudoa rosenbuschi* (Gelormini, 1944), *Microsporidium ovoideum* (Thélohan, 1895), *Myxidium baueri* Kovaleva et Gaevskaya, 1982, et *Myxoproteus meridianalis* Evdokimova, 1977; les métacestodes *Grillotia* sp. et *Hepatoxylon trichiuri* (Holten, 1802); enfin, le copépode parasite *Trifur tortuosus* Wilson, 1917. Le digénien adulte *Elytrophalloides oatesi* (Leiper et Atkinson, 1914) présentait un certain potentiel comme marque pour suivre les migrations saisonnières des merlus.

Introduction

The hake of the southwest Atlantic Ocean were considered to comprise the two species *Merluccius hubbsi* (Argentine hake) and *M. polylepis* (Patagonian hake) until Inada's (1981) study of morphometric and meristic characters resulted in *M. polylepis* being relegated to a junior synonym of *Merluccius australis* (New Zealand hake). Inada considered that the New Zealand and Patagonian hakes had not diverged sufficiently to warrant their recognition as separate species.

In the Atlantic, the geographical distributions of *M. hubbsi* and *M. australis* overlap around the Falkland Islands, while in the Pacific, the distribution of *M. australis* overlaps that of a third species, *Merluccius gayi* (Chilean hake), off the coast of southern Chile. Morphologically the three species are very similar and identification of individual

Received February 28, 1995. Accepted March 7, 1995.
J12807

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fish is difficult. Important commercial fisheries are based on all three species, but little is known about their population biologies. The hake fishery in the waters around the Falkland Islands is particularly important to the economy of the islands, but no nursery grounds have been identified in the area, and the migratory ranges of the *M. hubbsi* and *M. australis* caught on the Falklands Shelf are unknown.

The purpose of this study was to survey the parasite faunas of *M. australis* and *M. hubbsi* in the waters around and adjacent to the Falkland Islands and to assess the feasability of using some of these parasites as biological tags to identify different stocks and to follow the migrations of hake populations in the area.

Parasites have been used as biological tags to distinguish stocks of many species of both marine and freshwater fishes (see reviews by Sindermann (1961, 1983); Kabata (1963); MacKenzie (1983, 1987*a*); Lester (1990); Moser (1991); Williams et al. (1992)). Guidelines to the selection of parasite species likely to make the best tags are given in these reviews.

Materials and methods

Samples of hake from the Atlantic part of the study area were caught by trawl, while the Chilean sample was caught by long-line. Because the samples were taken by commercial fishing vessels and only small numbers of hake were usually caught in individual hauls, it was not possible to follow a pre-planned sampling program with any degree of accuracy. The samples detailed in Table 1 are therefore what were available to us from the areas where the fishing vessels were operating at the time. The fish were deep-frozen at sea and transported by sea and road to the SOAFD Marine Laboratory in Aberdeen, where they were defrosted. Individual fish were identified to species according to the criteria of Cousseau and Cotrina (1980) and Inada (1981). These identifications were subsequently checked against biochemical analyses of tissue samples using enzyme electrophoresis (J. Murphy, I. Wilson, and E. Verspoor, SOAFD Marine Laboratory, Aberdeen, Scotland, unpublished data). The length of each fish was measured to the nearest centimetre. Eleven samples of *M. australis* (113 fish) came from southern Chile (1) and the Falklands Shelf (10). Thirteen samples of *M. hubbsi* (195 fish) came from Argentinian waters (4) and from the Falklands Shelf (9). Sampling positions are shown in Fig. 1 and sampling details given in Table 1.

All the external surfaces of the fish, including the buccal and opercular cavities, were examined for macroparasites using a dissecting microscope at 10× magnification. The visceral cavity was then opened, the internal organs removed, and each examined for macroparasites using a dissecting microscope. The stomach and intestine were opened longitudinally and examined. Smears were taken from all organs and examined for protozoan parasites at magnifications of 300–500×. Representative samples of all parasite species found were preserved in either 8% buffered formal–saline or 70% ethanol and voucher specimens were deposited at the Natural History Museum in London, U.K.

The measures of level of infection used were (*i*) prevalence, defined as the number of fish infected by a particular parasite species divided by the total number of fish in the sample, expressed as a percentage, and (*ii*) abundance, defined as the total number of individuals of a particular parasite species in a sample of hosts divided by the total number of individuals of the host species in the sample (see Margolis et al. 1982).

Results

Twenty-eight parasite taxa were found in the two species of hake, of which 19 were identified to species level (Table 2). Also recorded were cysts of unknown etiology (CUEs) in the gill filaments. The myxosporean protozoan *Alatospora merluccii* Kalavati, Longshaw and MacKenzie, 1995 was found for the first time during the present study and was described by Kalavati et al. (1995). *Aporocotyle australis* Fernandez and Durán, 1985 and a single metacercaria of *Zoogonus* sp. were found only in *M. australis*. *Aporocotyle argentinensis* Smith, 1969 was found only in *M. hubbsi*, as was the single specimen of *Echinorhynchus* sp., which is also a new finding for this host. *Goussia* sp., *Lecithochirium genypteri* Manter, 1954, and *Gonocerca phycidis* Manter, 1925 are new findings for both species of hake, while *Myxoproteus meridianalis* Evdokimova, 1977, *Microsporidium ovoideum* (Thélohan, 1895); and *Zoogonus* sp. have not been previously reported from *M. australis*.

The hake samples examined in the present study were characterized by a predominance of one sex, usually females (258 females to 50 males in total), except for the Chilean sample of *M. australis*, which consisted of 23 males and 6 females. It was therefore not possible to carry out a meaningful comparison between the parasite faunas of the two sexes in any individual sample. Most of the samples contained less than 10 individuals of each hake species and showed large variations in mean length and length range. Tests of statistical significance on differences in prevalence and abundance between samples were therefore considered to be inappropriate.

Merluccius hubbsi (Tables 3–5)

Of the 26 parasites and pathological conditions found, several showed marked variations in prevalence and abundance between samples, particularly between those from the Falklands study area to the south and the Argentinian to the north. Fungal infections were relatively common around the Falklands, but only one fish from the Argentinian samples was infected. The only finding of *Alatospora merluccii* from *M. hubbsi* was in a fish caught to the east of the Falklands, while the other gall bladder myxosporean, *Myxidium baueri* Kovaleva and Gaevskaya, 1982, was found only around the Falklands. In contrast, the four other protozoan species were present only in samples taken to the west and north of the Falklands and in Argentinian waters. The blood-fluke *A. argentinensis* was most common in the Argentinian samples and was absent from those taken to the east and south of the Falkland Islands. Samples 1 and 3, taken 3 weeks apart from positions within 3 miles of one another, showed a remarkable difference in prevalence and abundance of the digenean *Elytrophalloides oatesi* (Leiper

Table 1. Details of hake samples in chronological order.

Sample No.	Date	No. of fish	Depth (m)	Length of fish (cm)		
				Range	Mean	SD
1	07/04/92	7A	300	53.6–72.7	66.0	6.3
		8H		51.2–74.5	60.3	7.0
2	11/04/92	13A	300	59.5–75.1	68.4	5.1
3	28/04/92	6A	262	58.4–77.0	65.6	8.1
		8H		48.9–63.0	53.9	4.5
4	07/92	29A	200–300	51.0–88.0	64.8	9.1
5	16/08/92	5H	308	55.7–61.6	58.6	2.8
6	16/08/92	3A	Unknown	69.0–75.0	71.3	3.2
7	16/08/92	6H	Unknown	61.4–73.2	69.0	4.6
8	22/08/92	20A	Unknown	60.4–74.1	68.2	4.2
9	04/10/92	7A	300	62.0–79.0	69.8	6.4
		3H		48.7–68.0	59.8	10.0
10	05/10/92	7A	361	50.0–76.0	65.4	10.6
		8H		42.5–71.5	55.3	11.3
11	07/10/92	10A	225	49.6–65.0	55.1	5.0
		30H		42.9–76.2	52.3	6.6
12	08/10/92	2A	131	42.3–49.3	45.8	5.0
		3H		47.0–56.6	52.5	5.0
13	10/10/92	9A	168	52.4–73.0	61.8	7.6
		37H		50.4–79.0	62.8	8.4
14	30/04/93	19H	Unknown	36.7–74.9	52.9	9.6
15	05/05/93	14H	Unknown	32.0–63.2	49.5	9.1
16	05/93	22H	Unknown	34.0–59.7	48.0	6.5
17	05/93	32H	Unknown	30.9–62.9	46.0	7.3

Note: A, *Merluccius australis*; H, *Merluccius hubbsi*. See Fig. 1 for sampling positions.

and Atkinson, 1914). Sample 1 was the only sample with no *E. oatesi*, whereas all eight fish in sample 3 were infected with from 10 to 73 parasites each (Table 3). All the cestodes, except for phyllobothriid plerocercoids, and the nematodes, were more common in the Falklands samples. Prevalence and abundance of CUEs were markedly greater in the two southern-most Argentinian samples than elsewhere.

***Merluccius australis* (Tables 6–7)**

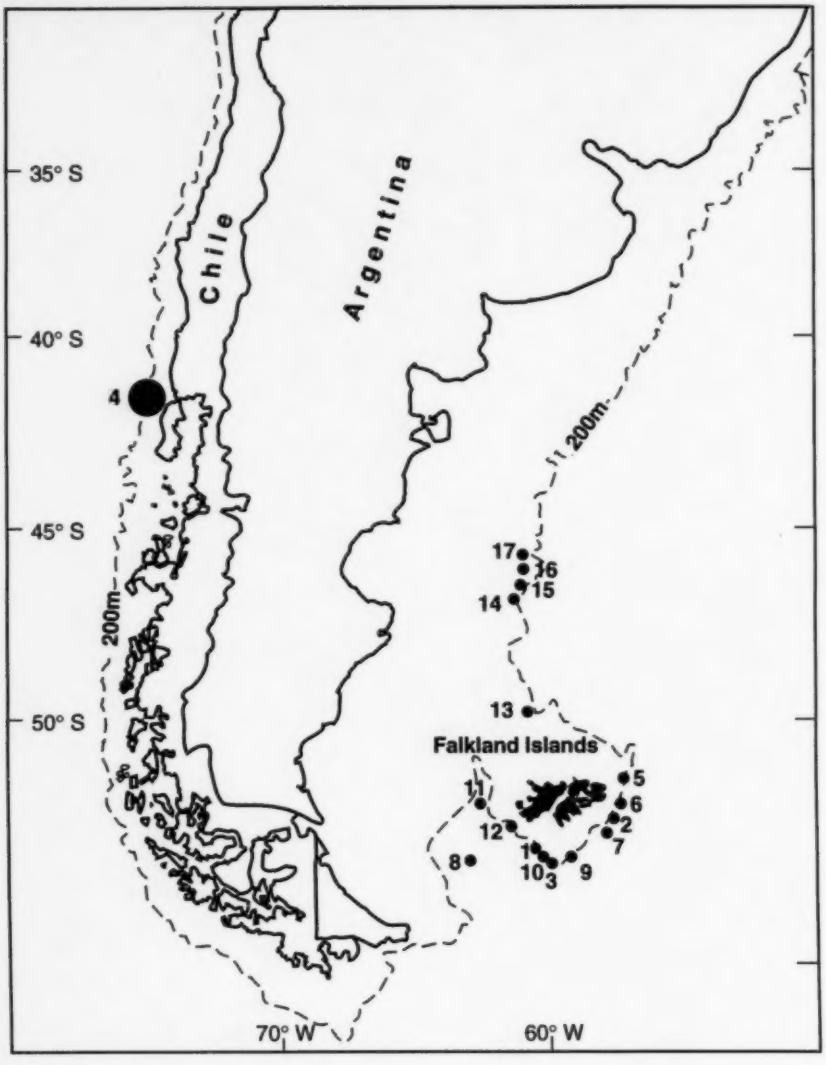
Twenty-seven parasite species and pathological conditions were recorded from *M. australis*. Some of the parasites showed apparent host preferences and discontinuous geographical distributions. *Alatospora merluccii* was much more common in *M. australis* than in *M. hubbsi* and was the only protozoan recorded from the Chilean sample, whereas *Goussia* sp. was much less common in *M. australis*. *Microsporidium ovoideum* was also much less common in *M. australis* than in *M. hubbsi* and was only found in samples from the north and west of the Falklands. *Anthocotyle merluccii* van Beneden and Hesse, 1863 was found only in the Chilean sample, while *Lacistorhynchus* sp. was found in only two fish, one from Chile and one from southwest of the Falkland Islands. Sample 1 contrasted markedly with sample 3 in prevalence and intensity of *E. oatesi* (Table 7), although the difference was not so great as in *M. hubbsi* from the same samples. Samples 1 and 4 (Chile) were the only samples without *E. oatesi*. The adult cestode *Clestobothrium crassiceps* (Rudolphi, 1819) was much more common in *M. australis* than in *M. hubbsi*.

Discussion

The parasite faunas of these two sympatric species of hake are very similar, with 23 of the 28 parasite taxa recorded in this study being common to both host species. One more parasite species was recorded from *Merluccius australis* than from *M. hubbsi*, although fewer *M. australis* were examined. More parasite species were also recorded from *M. hubbsi* caught around the Falkland Islands than from Argentinian waters. Both host species were caught around the Falkland Islands, but only *M. hubbsi* was present in the Argentinian samples, so the differences between their parasite faunas may be due more to differences in the geographical distributions of the parasites than to differences in host biology. There is evidence that male and female hake have different post-spawning migratory patterns in some areas (Di Giacomo et al. 1993), but the pronounced sex bias in our samples prevented us from testing for differences in parasite faunas between the sexes.

The fungal infection was found in hake from Chile and all Falklands areas except the west, but only one fish from the Argentine samples was infected. The infection is likely to be of long duration and it may prove to be a useful tag for *M. hubbsi*.

Fig. 1. Hake sampling positions.



All six protozoan species appear to have restricted distributions within the study area and all are potentially useful biological tags. The four myxosporeans are easily identified by their distinctive spore shapes and some myxosporean infections are known to persist for years (Lom and Dyková 1992). All are site specific; although *Kudoa rosenbuschi* (Gelormini, 1944) can occur anywhere in the musculature it is easily seen because of the black pigment associated with it.

Alatospora merluccii and *M. baueri* both appear to have southern distributions in the Atlantic study area, while *Goussia* sp., *K. rosenbuschi*, *M. ovoideum*, and *M. meridianalis* appear to have northern distributions. *Kudoa rosenbuschi* may have a northern distribution because Sardella (1988) found it in *M. hubbsi* from northern Argentine waters, whereas neither Reimer and Jessen (1981) nor Stott (1982) found it in *M. hubbsi* or *M. australis* from Falkland waters. Okada et al. (1980) found *Kudoa* sp. to be much more common in *M. gayi* from the more northern of two sampling stations along the coast of Peru, prevalences of infection being 40 and 14.4%. We did not find *Kudoa* in our Chilean sample of *M. australis*.

The *Goussia* sp. found in the livers of both species of hake is similar to *Goussia clupearum* (Thélohan, 1895). Morrison and Marryatt (1990) found a coccidian that they considered might be *G. clupearum* in the livers of *Merluccius bilinearis* in the northeast Atlantic, but our record may be the first of this coccidian from the Southern

Table 2. Parasites and pathological conditions recorded from *Merluccius australis* (Ma) and *Merluccius hubbsi* (Mh).

Parasite	Site of infection	Host species		Voucher specimen No.
		Ma	Mh	
Fungus				
Unidentified fungus	All soft tissues, especially liver	+	+	None
Protozoa				
Alatospora merluccii Kalavati, Longshaw, and MacKenzie, 1995	Gall bladder	+	+	1994:3:15:1
Goussia sp.	Liver	+	+	None
Kudoa rosenbuschi (Gelormini, 1944)	Musculature	+	+	1994:6:13:3
Microsporidium ovoideum (Thélohan, 1895)	Liver	+	+	1994:6:13:1
Myxidium baueri Kovaleva and Gaevskaya, 1982	Gall bladder	+	+	1994:3:15:2
Myxoproteus meridianalis Evdokimova, 1977	Urinary bladder	+	+	1994:6:13:2
Monogenea				
Anthocotyle merluccii van Beneden and Hesse, 1863	Gills	+	+	1994.6.7.66–68
Digenea				
Aporocotyle argentinensis Smith, 1969	Heart, blood vessels	—	+	1994.6.7.30–34
A. australis Fernandez and Durán, 1985	Heart, blood vessels	+	—	1994.6.7.35
Derogenes varicus (O.F. Müller, 1784)	Stomach, oesophagus, buccal cavity	+	+	1994.6.7.25–29
Elytrophalloides oatesi (Leiper and Atkinson, 1914)	Stomach, oesophagus, buccal cavity	+	+	1994.6.7.10–19
Gonocerca phycidis Manter, 1925	Stomach, oesophagus, buccal cavity	+	+	1994.6.7.20–24
Lecithochirium genypteri Manter, 1954	Stomach wall	+	+	1994.6.7.1–9
Zoogonus sp. metacercaria	Liver	+	—	None
Cestoda				
Clestobothrium crassiceps (Rudolphi, 1819)	Intestine	+	+	1994.6.7.36
Grillotia sp. plerocercoid	Visceral cavity, stomach wall	+	+	1994.6.7.42–43
Hepatoxylon trichiuri (Holten, 1802) plerocercoid	Visceral cavity	+	+	1994.6.7.38–41
Lacistorhynchus sp. plerocercoid	Musculature	+	—	None
Diphyllobothriidae plerocercoid	Stomach wall	+	+	1994.6.7.64–65
Phyllobothriidae plerocercoid	Intestine	+	+	1994.6.7.44–63
Nematoda				
Anisakis simplex (Rudolphi, 1809) larva	Visceral cavity	+	+	None
Hysterothylacium aduncum (Rudolphi, 1802)	Stomach, intestine	+	+	None
Hysterothylacium and *Contracaecum* larva	Stomach, intestine	+	+	None
Acanthocephala				
Echinorhynchus sp.	Intestine	—	+	None
Copepoda				
Chondracanthus palpifer Wilson, 1912	Buccal cavity	+	+	1994.3291–3301
Neobrachiella insidiosa f. *lageniformis* Kabata, 1979	Gills	+	+	1994.3304–3313
Trifur tortuosus Wilson, 1917	Body surface	+	+	1994.3302–3303
Other pathological conditions				
Cysts of unknown etiology (CUEs)	Gill filaments	+	+	None

Hemisphere. *Myxidium baueri* was originally described from *Coryphaenoides holotrachys* and *M. hubbsi* caught in the southwest Atlantic by Kovaleva and Gaevskaya (1982). Kalavati et al. (1995) drew attention to some anomalies between their observations and the original description and suggested that material from the two hake species and *C. holotrachys* requires further comparative study to determine if one or more species of *Myxidium* is involved.

The only monogenean found on these hake, *Anthocotyle merluccii*, was not particularly common at any of the sampling locations but was widely distributed in *M. hubbsi*. In *M. australis*, however, it was only found in the Chilean

Table 3. Prevalence (*P*, %) and abundance (*A*, metazoan parasites only) of parasites and CUEs in samples of *Merluccius hubbsi* from east and south of the Falkland Islands (see Fig. 1 and Table 1).

	Sample 1 (n = 8)			Sample 3 (n = 8)			Sample 5 (n = 5)			Sample 7 (n = 6)			Sample 9 (n = 3)		
		A			*A*			*A*			*A*			*A*	
Parasite	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range
Fungus															
Unidentified fungus	63	—	—	25	—	—	0	—	—	33	—	—	0	—	—
Protozoa															
Alatospora merluccii	0	—	—	0	—	—	20	—	—	0	—	—	0	—	—
Myxidium baueri	12	—	—	12	—	—	0	—	—	17	—	—	33	—	—
Monogenea															
Anthocotyle merluccii	12	0.1±0.4	0–1	12	0.1±0.4	0–1	0	0	0	0	0	0	33	0.3±0.6	0–1
Digenea															
Derogenes varicus	63	3.4±4.3	0–11	88	6.4±5.6	0–18	40	4.0±5.7	0–12	100	7.0±6.2	1–17	67	3.0±2.7	0–5
Elytrophalloides oatesi	0	0	0	100	36.9±20.3	10–73	60	3.8±5.9	0–14	67	17.2±22.2	0–60	100	6.7±3.2	3–9
Gonocerca phycidis	0	0	0	0	0	0	0	0	0	17	0.2±0.4	0–1	33	1.0±1.7	0–3
Cestoda															
Clestobothrium crassiceps	0	0	0	0	0	0	0	0	0	17	0.2±0.4	0–1	0	0	0
Grillotia sp.	50	1.4±2.1	0–6	25	0.4±0.7	0–2	80	1.8±1.3	0–3	50	3.3±5.5	0–14	33	1.3±2.3	0–4
Hepatoxylon trichiuri	25	0.3±0.5	0–1	0	0	0	0	0	0	17	0.2±0.4	0–1	33	0.7±1.2	0–2
Diphyllobothriidae	0	0	0	12	0.1±0.4	0–1	20	0.2±0.4	0–1	17	0.5±1.2	0–3	67	1.0±1.0	0–2
Phyllobothriidae[a]	0	—	—	0	—	—	0	—	—	0	—	—	100	—	—
Nematoda															
Anisakis simplex	100	23.1±5.4	16–31	100	17.1±8.6	7–28	100	16.0±5.5	8–22	100	23.8±14.3	10–42	67	8.3±12.7	0–23
Hysterothylacium + *Contracaecum* spp.	50	2.0±2.4	0–6	88	6.8±4.4	0–13	100	10.6±14.1	1–34	100	23.2±21.7	1–48	67	2.7±3.1	0–6
Copepoda															
Chondracanthus palpifer	63	1.5±1.9	0–5	25	0.6±1.2	0–3	20	0.4±0.9	0–2	83	4.3±8.2	0–21	0	0	0
Neobrachiella insidiosa f. *lageniformis*	12	0.1±0.4	0–1	25	0.5±1.1	0–3	20	0.6±1.3	0–3	17	0.2±0.4	0–1	0	0	0
Trifur tortuosus	12	0.1±0.4	0–1	12	0.1±0.4	0–1	0	0	0	0	0	0	33	0.3±0.6	0–1
Others															
CUEs	38	1.5±2.3	0–6	0	0	0	0	0	0	0	0	0	67	2.3±3.2	0–6

[a]Numbers of Phyllobothriidae were not counted.

Table 4. Prevalence (*P*, %) and abundance (*A*, metazoan parasites only) of parasites and CUEs in samples of *Merluccius hubbsi* from south, west, and north of the Falkland Islands (see Fig. 1 and Table 1).

	Sample 10 (n = 8)			Sample 11 (n = 30)			Sample 12 (n = 3)			Sample 13 (n = 37)		
		A			*A*			*A*			*A*	
Parasite	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range
Fungus												
Unidentified fungus	37	—	—	0	—	—	0	—	—	19	—	—
Protozoa												
Goussia sp.	0	—	—	0	—	—	0	—	—	3	—	—
Kudoa rosenbuschi	0	—	—	13	—	—	0	—	—	11	—	—
Microsporidium ovoideum	0	—	—	33	—	—	0	—	—	22	—	—
Myxidium baueri	12	—	—	0	—	—	0	—	—	3	—	—
Myxoproteus meridianalis	0	—	—	43	—	—	0	—	—	16	—	—
Monogenea												
Anthocotyle merluccii	0	0	0	13	0.1±0.4	0–2	0	0	0	16	0.2±0.6	0–3
Digenea												
Aporocotyle argentinensis	0	0	0	3	0.03±0.2	0–1	0	0	0	3	0.02±0.2	0–1
Derogenes varicus	100	5.4±3.3	1–10	73	3.7±3.7	0–12	100	5.7±5.0	1–11	65	2.4±3.8	0–18
Elytrophalloides oatesi	100	3.5±3.1	1–10	80	6.6±7.3	0–26	100	8.7±5.5	3–14	68	6.1±8.8	0–34
Gonocerca phycidis	25	0.5±0.9	0–2	0	0	0	33	0.7±1.2	0–2	11	0.2±0.5	0–2
Lecithochirium genypteri	12	2.5±7.1	0–20	7	0.1±0.4	0–2	33	0.3±0.6	0–1	16	1.3±4.8	0–24
Cestoda												
Clestobothrium crassiceps	0	0	0	0	0	0	33	1.7±2.9	0–5	0	0	0
Grillotia sp.	50	1.6±2.3	0–6	53	2.5±5.3	0–25	33	0.3±0.6	0–1	38	1.8±3.8	0–17
Hepatoxylon trichiuri	12	0.1±0.4	0–1	3	0.03±0.2	0–1	0	0	0	24	0.6±1.2	0–4
Diphyllobothriidae	50	2.1±3.6	0–10	33	1.3±3.7	0–20	33	1.0±1.7	0–3	35	2.5±7.1	0–40
Phyllobothriidae[a]	12	—	—	7	—	—	0	—	—	14	—	—
Nematoda												
Anisakis simplex	100	27.3±33.4	1–107	97	11.5±10.4	0–38	67	8.0±6.9	0–12	100	17.7±15.2	4–78
Hysterothylacium + *Contracaecum* spp.	50	8.0±14.0	0–41	70	7.1±11.5	0–50	67	5.7±8.1	0–15	79	11.2±17.0	0–76
Acanthocephala												
Echinorhynchus sp.	0	0	0	0	0	0	0	0	0	3	0.02±0.2	0–1
Copepoda												
Chondracanthus palpifer	25	0.6±1.2	0–3	37	0.5±0.9	0–4	33	0.3±0.6	0–1	49	1.1±2.2	0–13
Neobrachiella insidiosa. f. *lageniformis*	50	0.9±1.1	0–3	23	1.3±4.9	0–7	0	0	0	16	0.3±0.9	0–4
Trifur tortuosus	12	0.1±0.4	0–1	0	0	0	0	0	0	0	0	0
Others												
CUEs	25	0.6±1.2	0–3	17	1.1±3.8	0–20	33	0.7±1.2	0–2	14	0.2±0.4	0–2

[a]Numbers of Phyllobothriidae were not counted.

Table 5. Prevalence (*P*, %) and abundance (*A*, metazoan parasites only) of parasites and CUEs in samples of *Merluccius hubbsi* from Argentine waters (see Fig. 1 and Table 1).

	Sample 14 (n = 19)			Sample 15 (n = 14)			Sample 16 (n = 22)			Sample 17 (n = 32)		
		A			*A*			*A*			*A*	
Parasite	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range
Fungus												
Unidentified fungus	5	—	—	0	—	—	0	—	—	0	—	—
Protozoa												
Goussia sp.	47	—	—	36	—	—	23	—	—	31	—	—
Kudoa rosenbuschi	26	—	—	43	—	—	9	—	—	25	—	—
Microsporidium ovoideum	63	—	—	36	—	—	41	—	—	16	—	—
Myxoproteus meridianalis	5	—	—	14	—	—	14	—	—	19	—	—
Monogenea												
Anthocotyle merluccii	11	0.2±0.7	0–3	7	0.1±0.3	0–1	9	0.1±0.3	0–1	3	0.03±0.2	0–1
Digenea												
Aporocotyle argentinensis	26	0.6±1.3	0–5	14	0.1±0.4	0–1	46	0.7±1.4	0–5	38	1.1±2.3	0–11
Derogenes varicus	63	3.1±5.1	0–22	57	2.3±4.0	0–15	68	2.4±2.4	0–8	47	1.0±1.5	0–6
Elytrophalloides oatesi	58	3.8±6.0	0–20	64	1.3±1.4	0–5	50	2.4±4.8	0–21	25	1.3±2.8	0–10
Gonocerca phycidis	11	0.2±0.7	0–3	0	0	0	18	0.2±0.4	0–1	3	0.3±0.2	0–1
Lecithochirium genypteri	0	0	0	7	0.3±1.1	0–4	0	0	0	6	0.1±0.2	0–1
Cestoda												
Grillotia sp.	26	0.3±0.6	0–2	21	0.3±0.6	0–2	0	0	0	3	0.03±0.2	0–1
Hepatoxylon trichiuri	16	0.2±0.4	0–1	0	0	0	0	0	0	0	0	0
Diphyllobothriidae	26	4.8±13.2	0–55	29	1.0±2.2	0–8	14	0.1±0.4	0–1	6	0.7±3.6	0–20
Phyllobothriidae[a]	47	—	—	43	—	—	14	—	—	16	—	—
Nematoda												
Anisakis simplex	100	7.7±6.4	1–26	93	9.9±8.8	0–30	82	8.0±8.2	0–30	78	4.7±5.1	0–25
Hysterothylacium + *Contracaecum* spp.	58	3.2±4.0	0–14	43	3.6±6.0	0–17	46	4.3±9.0	0–41	47	2.8±6.4	0–33
Copepoda												
Chondracanthus palpifer	37	0.6±1.0	0–4	36	0.6±1.1	0–3	32	0.6±1.2	0–4	25	0.4±0.8	0–3
Neobrachiella insidiosa. f. *lageniformis*	21	0.5±1.1	0–4	29	0.7±1.4	0–4	18	0.2±0.5	0–2	25	0.5±1.2	0–6
Trifur tortuosus	0	0	0	0	0	0	0	0	0	3	0.03±0.2	0–1
Others												
CUEs	84	6.0±7.3	0–25	43	2.3±4.8	0–18	9	0.2±0.7	0–3	13	0.2±0.6	0–3

[a]Numbers of Phyllobothriidae were not counted.

sample. Fernandez (1985) found 43% prevalence of *A. merluccii* in a sample of *M. australis* caught close to our Chilean sampling area, from which we recorded only 10% prevalence. Reimer and Jessen (1981) did not report *A. merluccii* in *M. hubbsi* from their southwest Atlantic study area, but Suriano and Martorelli (1984) found it in *M. hubbsi* from two localities on the Argentine shelf, both north of the Falklands. The evidence suggests a northern distribution of *A. merluccii* within our study area, so it may have some potential as a tag for *M. australis*.

Among the digeneans, *Elytrophalloides oatesi* stands out because of its occurrence in 15 of the 16 Atlantic samples, and the marked contrast between sample 1, the sole uninfected sample, and the heavily infected sample 3 (Tables 3 and 7). These samples were taken 3 weeks apart from virtually the same position and the fish were of comparable size and had similar sexual compositions. Although the numbers of fish in these samples were small, the marked difference between them in prevalence and abundance of *E. oatesi* indicates that either the samples were taken from different populations of hake or that the infections were acquired by the same population in the period between the two sampling dates. Nonetheless, *E. oatesi* is worth noting as a potentially useful tag for following seasonal migrations of both species of hake. *Aporocotyle argentinensis* appears to have a northern distribution and may have some potential as a tag for *M. hubbsi*.

We identified the species of *Gonocerca* from our hake as *G. phycidis*, but Gaevskaya and Kovaleva (1978) reported *G. crassa* Manter, 1934 from *M. hubbsi*. Gibson (1976) thought it likely that *G. crassa* is conspecific with *G. phycidis*, and Gibson and Bray (1979) considered that any change in the taxonomic status of these two species and the very similar *Derogenes varicus* (O.F. Müller, 1784) should await a complete redescription.

Of the Cestoda encountered, *Grillotia* sp. plerocercoids showed evidence of a southern distribution in the Atlantic study area and were not found in the Chilean sample, although *Grillotia heptanchi* (Vaullegeard, 1899) plerocercoids were reported at prevalences of about 16% by Carvajal and Campbell (1979) and Fernandez (1985) from *M. australis* caught within our Chilean sampling area. The material we examined had been frozen and we could not identify the plerocercoids to the specific level because we were unable to evert the tentacles. The plerocercoid is easily detected and related species are known to have life spans of several years in their fish hosts (MacKenzie 1987*b*).

The plerocercoid of *Hepatoxylon trichiuri* Holten, 1802 is a large, easily detected worm. It does not show such a clear trend as *Grillotia* sp. in its geographical distribution, being found in about 15% of hake caught around the Falklands but present in only the southern-most of the Argentine samples. This suggests that it may prove to be a useful tag for *M. hubbsi* over a study area extending further north. We found it in only 3% of our Chilean *M. australis*, but Fernandez (1985) found it in 34% of her sample from this area.

The adult cestode *Clestobothrium crassiceps* appears to have a southern distribution in our Atlantic study area. Only two individuals of *M. hubbsi* were infected, both caught near the limits of this host's range to the south of the Falklands. On the Pacific side it also seems to have a southern distribution and is much more common in *M. australis* than in *M. gayi* (Carvajal et al. 1979; Durán and Oliva 1980; Fernandez 1985). The life span of *C. crassiceps* is unknown, but it is a large easily detected worm that shows some promise as a biological tag.

Neither the phyllobothriid nor the diphyllobothriid plerocercoids showed any clear pattern of geographical distribution. The phyllobothriid plerocercoids are among those commonly referred to under the collective name *Scolex pleuronectis* O.F. Müller, 1788, a term that embraces a number of tetraphyllidean taxa which are difficult to separate at this stage of development.

Of the Nematoda, *Anisakis simplex* (Rudolphi, 1809) was uniformly common in all areas and showed no marked variations in prevalence, although the variations in abundance may be significant. To use these nematodes as tags, however, would necessitate detecting and counting every individual scattered throughout the tissues of the host, which is very time consuming. We did not distinguish between the genera *Hysterothylacium* and *Contracaecum* because to identify each individual of such abundant worms would also have been prohibitively time consuming.

Of the three copepod species infecting these hake, *Trifur tortuosus* Wilson, 1917 shows most potential as a tag. It was not common in any area but was particularly rare to the north and west of the Falklands, where it was found in only one fish from an Argentine sample. A particular advantage of this species as a tag is that it is a large easily seen ectoparasite that leaves a prominent scar after it dies, thereby extending its usefulness as a tag beyond its actual life span.

The CUEs showed wide local variations in prevalence between adjacent samples and further sampling may show them to have some value as tags.

Conclusions

A survey such as this, covering a wide area, with samples taken at different times of the year and consisting of small numbers of fish of mixed sizes and ages, cannot lead to firm conclusions with regard to stock identification and migrations of the host species. It can, however, provide a basis for assessing the feasability of a biological tag study and for planning more focused sampling programs. The results highlight a number of parasite species as being potentially useful for stock identification, where long life spans are important. These are the fungus, the six protozoan species, the cestodes *Grillotia* sp. and *H. trichiuri*, and the copepod *T. tortuosus*.

Some specific suggestions for using hake parasites as biological tags can be made from the results of this study. One possible reason for the marked differences between the parasite faunas of *M. hubbsi* from the Falklands Shelf and those from Argentine waters further north is that these populations do not mix to any great extent. A more intensive sampling program concentrating on those parasites showing the most marked southern or northern distributions in the Atlantic study area could be designed to show if such mixing does take place and to what extent. These

Table 6. Prevalence (*P*, %) and abundance (*A*, metazoan parasites only) of parasites and CUEs in samples of *Merluccius australis* from Chile and west and north of the Falkland Islands (see Fig. 1 and Table 1).

	Sample 4 (n = 29)			Sample 8 (n = 20)			Sample 11 + 12 (n = 12)			Sample 13 (n = 9)		
		A			*A*			*A*			*A*	
Parasite	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range
Fungus												
Unidentified fungus	38	—	—	25	—	—	0	—	—	11	—	—
Protozoa												
Alatospora merluccii	21	—	—	20	—	—	0	—	—	0	—	—
Goussia sp.	0	—	—	5	—	—	0	—	—	11	—	—
Kudoa rosenbuschi	0	—	—	20	—	—	8	—	—	44	—	—
Myxidium baueri	0	—	—	65	—	—	0	—	—	0	—	—
Microsporidium ovoideum	0	—	—	5	—	—	42	—	—	33	—	—
Myxoproteus meridianalis	0	—	—	20	—	—	33	—	—	44	—	—
Monogenea												
Anthocotyle merluccii	10	0.1±0.4	0–2	0	0	0	0	0	0	0	0	0
Digenea												
Aporocotyle australis	62	2.8±5.8	0–27	0	0	0	0	0	0	22	0.2±0.4	0–1
Derogenes varicus	3	0.03±0.2	0–1	35	0.7±1.1	0–3	75	3.9±4.3	0–13	56	2.0±2.9	0–9
Elytrophalloides oatesi	0	0	0	85	7.2±8.9	0–40	75	6.9±7.4	0–19	78	5.3±6.1	0–16
Gonocerca phycidis	0	0	0	5	0.03±0.2	0–1	0	0	0	11	0.1±0.3	0–1
Lecithochirium genypteri	0	0	0	0	0	0	0	0	0	11	0.1±0.3	0–1
Zoogonus sp.	0	0	0	0	0	0	8	0.1±0.3	0–1	0	0	0
Cestoda												
Clestobothrium crassiceps	90	16.8±17.6	0–54	0	0	0	8	0.1±0.3	0–1	11	0.1±0.3	0–1
Grillotia sp.	0	0	0	5	0.1±0.2	0–1	58	3.3±6.1	0–20	56	1.6±2.0	0–6
Hepatoxylon trichiuri	7	0.1±0.3	0–1	10	0.2±0.5	0–2	0	0	0	0	0	0
Lacistorhynchus sp.	3	0.1±0.4	0–2	5	0.1±0.2	0–1	0	0	0	0	0	0
Diphyllobothriidae	7	0.1±0.4	0–2	10	0.3±0.8	0–3	67	5.7±11.1	0–40	44	1.1±1.7	0–4
Phyllobothriidae[a]	21	—	—	5	—	—	8	—	—	44	—	—
Nematoda												
Anisakis simplex	97	14.8±21.7	0–98	100	14.4±9.3	4–33	100	10.8±6.8	2–29	89	19.2±19.9	0–53
Hysterothylacium + *Contracaecum* spp.	38	8.3±19.0	0–80	75	7.7±10.0	0–40	67	3.6±3.9	0–10	56	3.8±5.6	0–16
Copepoda												
Chondracanthus palpifer	14	0.2±0.5	0–2	0	0	0	25	0.3±0.5	0–1	33	1.0±2.0	0–6
Neobrachiella insidiosa. f. *lageniformis*	24	0.8±2.5	0–13	45	0.9±1.4	0–5	17	0.4±1.0	0–3	11	0.1±0.3	0–1
Trifur tortuosus	3	0.03±0.2	0–1	0	0	0	8	0.2±0.6	0–2	0	0	0
Others												
CUEs	90	4.9±3.9	0–13	75	8.6±8.7	0–30	25	0.3±0.5	0–1	22	0.6±1.1	0–3

Note: Samples 11 and 12 were combined because they were taken on consecutive days from positions close to one another and sample 12 included only two *M. australis*.
[a]Numbers of Phyllobothriidae were not counted.

Table 7. Prevalence (*P*, %) and abundance (*A*, metazoan parasites only) of parasites and CUEs in samples of *Merluccius australis* from east and south of the Falkland Islands (see Fig. 1 and Table 1).

	Sample 1 (n = 7)			Sample 2 (n = 13)			Sample 3 (n = 6)			Sample 6 (n = 3)			Sample 9 + 10 (n = 14)		
		A			*A*			*A*			*A*			*A*	
Parasite	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range	*P*	$\bar{x}$ ± SD	Range
Fungus															
Unidentified fungus	57	—	—	46	—	—	33	—	—	33	—	—	14	—	—
Protozoa															
Alatospora merluccii	0	—	—	8	—	—	0	—	—	0	—	—	7	—	—
Kudoa rosenbuschi	0	—	—	0	—	—	0	—	—	33	—	—	7	—	—
Myxidium baueri	57	—	—	62	—	—	50	—	—	67	—	—	50	—	—
Myxoproteus meridianalis	0	—	—	0	—	—	0	—	—	67	—	—	21	—	—
Digenea															
Aporocotyle australis	0	0	0	8	0.5±1.7	0–6	0	0	0	0	0	0	7	0.1±0.3	0–1
Derogenes varicus	29	1.4±3.0	0–8	77	7.0±8.9	0–27	33	0.7±1.0	0–2	100	2.3±1.5	1–4	79	3.0±3.6	0–10
Elytrophalloides oatesi	0	0	0	54	5.2±11.2	0–40	67	3.7±3.4	0–8	100	22.0±24.8	3–50	79	16.6±17.4	0–51
Gonocerca phycidis	0	0	0	0	0	0	0	0	0	0	0	0	21	0.5±1.1	0–3
Lecithochirium genypteri	0	0	0	0	0	0	17	0.3±0.8	0–2	0	0	0	0	0	0
Cestoda															
Clestobothrium crassiceps	14	0.1±0.4	0–1	15	0.5±1.7	0–6	33	8.5±20.3	0–50	67	1.7±2.1	0–4	21	0.4±0.7	0–2
Grillotia sp.	14	0.1±0.4	0–2	8	0.2±0.5	0–2	17	0.2±0.4	0–1	0	0	0	7	0.2±0.8	0–3
Hepatoxylon trichiuri	14	0.3±0.8	0–2	15	0.2±0.4	0–1	17	0.2±0.4	0–1	0	0	0	36	0.5±0.9	0–3
Diphyllobothriidae	0	0	0	15	0.3±0.8	0–2	0	0	0	33	0.3±0.6	0–1	14	0.6±1.9	0–7
Phyllobothriidae[a]	14	—	—	31	—	—	17	—	—	33	—	—	21	—	—
Nematoda															
Anisakis simplex	86	11.4±8.6	0–22	100	13.3±5.2	7–22	100	16.8±7.4	7–27	100	23.0±14.7	14–40	100	14.6±12.1	3–46
Hysterothylacium + *Contracaecum* spp.	71	4.1±4.6	0–12	69	2.3±4.6	0–17	83	2.3±2.2	0–5	33	5.0±8.7	0–15	79	10.6±14.7	0–50
Copepoda															
Chondracanthus palpifer	0	0	0	8	0.1±0.3	0–1	17	0.2±0.4	0–1	0	0	0	7	0.1±0.3	0–1
Neobrachiella insidiosa f. *lageniformis*	43	0.7±1.0	0–2	62	1.8±1.8	0–5	17	1.3±3.3	0–8	67	1.3±1.5	0–3	36	1.2±2.3	0–8
Trifur tortuosus	0	0	0	8	0.1±0.3	0–1	0	0	0	0	0	0	7	0.1±0.3	0–1
Others															
CUEs	57	2.1±3.6	0–10	77	5.9±5.3	0–16	33	3.8±8.0	0–20	100	9.3±11.9	2–23	71	8.4±13.8	0–37

Note: Samples 9 and 10 were combined because they were taken on consecutive days from positions close to one another and the size ranges of the fish were comparable.
[a]Numbers of Phyllobothriidae were not counted.

parasites are *Alatospora merluccii*, *Myxidium baueri*, *Goussia* sp., *Aporocotyle argentinensis*, and *Clestobothrium crassiceps*. It is likely that *Anthocotyle merluccii*, *Elytrophalloides oatesi*, and all the protozoans except *A. merluccii* could be used to show the degree of mixing, if any, between Atlantic and Pacific populations of *M. australis*.

Acknowledgements

We are grateful to Mr. Peter Davy and Mr. Patrick Davy of Stevedore and Fishery Services Ltd. for funding this project. We thank Dr. Conor Nolan and staff of the Fisheries Department, Stanley, Falkland Islands, for obtaining samples of fish and providing laboratory facilities, and Professor A.D. Hawkins, Director, SOAFD Marine Laboratory, Aberdeen, Scotland, for providing laboratory facilities. We also thank Dr. R.A. Bray of the Natural History Museum, London, for identifying the species of *Lecithochirium* as *L. genypteri*.

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Stock composition and movement of gemfish, *Rexea solandri*, as indicated by parasites

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Abstract: The parasite fauna of gemfish, *Rexea solandri*, from seven areas off southern Australia, was examined for evidence of isolated gemfish populations. Canonical multivariate analyses of data on larval nematodes (*Anisakis* spp. and *Terranova* sp.), cestode plerocercoids (*Hepatoxylon trichiuri* and *Nybelinia* sp.), acanthocephalans (*Rhadinorhynchus* sp. and *Corynosoma* sp.), and a hemiuroid digenean from a total of 763 gemfish showed that the parasite faunas of fish from eastern Australia were similar except for a sample taken off New South Wales at the end of the spawning season whose affinities are unknown. Fish from South Australia had similar parasite faunas to those collected from eastern Australia, suggesting that fish from the eastern and western Bass Strait belong to the same stock. Fish collected from the Great Australian Bight were distinct from the southern and eastern fish. Differences in parasite fauna were detected between samples taken within the spawning season and those taken from the same locations outside the spawning season, presumably a result of the spawning migration.

Résumé : La faune parasite de l'escolier, *Rexea solandri*, de sept zones marines du sud de l'Australie a été examinée pour la recherche d'indices de l'existence de populations isolées de ce poisson. L'analyse canonique par variables multiples de données sur les nématodes (*Anisakis* spp. et *Terranova* sp.), les cestodes plérocercoïdes (*Hepatoxylon trichiuri* et *Nybelinia* sp.), les acanthocéphales (*Rhadinorhynchus* sp. et *Corynosoma* sp.) et un hémiuroïde digène prélevés de 763 escoliers a permis de montrer que les faunes parasites des poissons de l'est de l'Australie étaient semblables. La seule exception avait trait à un échantillon prélevé à la fin de la saison du frai au large des Nouvelles Galles du Sud et dont les affinités n'ont pu être déterminées. Les poissons capturés au large de la côte sud présentaient une faune parasite semblable à celle des poissons capturés à l'est, ce qui porte à croire que les poissons de l'est et de l'ouest du détroit Bass appartiennent au même stock. Les poissons prélevés dans la Grande Baie australienne différaient de ceux du sud et de l'est. Des écarts de faunes parasites ont été décelés entre les échantillons prélevés pendant la saison du frai et ceux prélevés hors cette saison, mais aux mêmes endroits; de tels écarts s'expliquent sans doute par la migration de frai.
[Traduit par la Rédaction]

Introduction

Gemfish, *Rexea solandri*, occur along the southeastern and southern Australian coastline and around New Zealand (Parin and Paxton 1990). They have supported commercial fisheries in these two countries (Hurst 1988; Rowling 1990). In Australia, the main fishery is along the southeast coast, with a smaller fishery to the west of Bass Strait. Spawning aggregations of gemfish that are believed to migrate up the southeast coast were taken by the southeastern trawl fishery from depths of 300–400 m from the early 1970s until 1993 (Rowling 1990; Joll 1993). Landings reached a peak of 5100 t in 1980 and continued close to this level for several years, but decreases in the catch per unit effort and in the average length of fish lead to fears of a collapse of the fishery (Kearney 1989; Rowling 1990). A zero quota was announced for 1993 (Joll 1993).

Larval gemfish were caught in plankton tows off the New South Wales coast north of Sydney, suggesting a spawning area was nearby (Dr. A. Miskiewicz, NSW Department of Agriculture, Sydney, personal communication). Current management procedures for the southeastern trawl fishery operate on the assumption that gemfish from southeastern Australian waters belong to a single unit stock reliant on the New South Wales spawning area, and that gemfish to the west of Bass Strait belong to a different stock (Kaloila et al. 1993; Lyle and Ford 1993). An artificial tag program to clarify the stock structure has not yet been attempted because gemfish captured in trawl nets and on drop lines arrive at the surface in poor condition (Rowling 1990).

Received December 12, 1994. Accepted April 27, 1995.
J12890

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Table 1. The origins of the 23 samples of gemfish examined.

Group	Sample number	Capture date	Number in sample	Position
1*a*	1	10 Nov. 1984	41	33°31′S, 129°30′E
	2	13 Nov. 1984	28	33°28′S, 130°57′E
	3	23 Nov. 1984	53	33°27′S, 126°58′E
	4	23 Nov. 1984	17	33°23′S, 128°03′E
1*b*	1	1 June 1985	15	33°32′S, 127°09′E
	2	2 June 1985	14	33°32′S, 129°00′E
	3	4 June 1985	15	33°33′S, 131°07′E
2	1	27 Nov. 1984	29	36°04′S, 135°46′E
3	1	2 Nov. 1984	48	37°52′S, 139°47′E
	2	2 Nov. 1984	23	37°11′S, 139°56′E
4*a*	1	15 Dec. 1984	50	43°38′S, 147°50′E
4*b*	1	23 Jan. 1985	8	East of Bruny Island
	2	9 July 1985	43	43°35′S, 147°57′E
	3	12 Aug. 1985	43	43°35′S, 147°56′E
4*c*	1	21 Jan. 1986	44	East of Bruny Island
5	1	27 Apr. 1985	16	42°40′S, 148°25′E
	2	22 Aug. 1985	29	42°42′S, 148°25′E
	3	8 Apr. 1986	14	42°41′S, 148°24′E
6	1	26 Mar. 1985	50	41°37′S, 148°37′E
7*a*	1	16 Sep. 1985	29	Off Wollongong
7*b*	1	3 July 1984	35	Off Sydney
7*c*	1	1 July 1987	63	Off Wollongong
7*d*	1	3 July 1987	56	Off Wollongong

Data on parasites have been used increasingly to draw conclusions about the distributions of fish populations since the earliest recorded use of parasites as indicators of host populations by Dogiel and Bykhovsky (1939) and Herrington et al. (1939) and the pioneering studies of Margolis (1957, 1965), Sindermann (1961*a*, 1961*b*, 1983), and Kabata (1963) (see MacKenzie 1983, 1987). Analysis of the numbers of parasites in individual fish from samples taken from different geographical areas can provide information on the extent of movement of fish between these areas, and thus provide evidence of isolated or semi-isolated fish populations, although parasites may be less useful to determine the precise boundaries of fish populations (Lester 1990). Sindermann (1983) suggested using several parasite species simultaneously if no one parasite was ideal. Multivariate analysis of several parasite species uses more data and can be more sensitive than analysis of data from a single species, thus offsetting the requirement of large sample sizes. Lester et al. (1985) used canonical multivariate analysis on parasite data from 800 skipjack tuna, *Katsuwonus pelamis*, from the Pacific Ocean to conclude that the fish sampled were part of a single large stock. Later, Lester et al. (1988) used the same methods to demonstrate that orange roughy, *Hoplostethus atlanticus*, from Australian and New Zealand waters comprised at least eight discrete stocks.

In the present study, univariate and canonical multivariate analyses were used on parasite data from gemfish primarily to determine the stock structure of gemfish from southern Australian waters.

Materials and methods

Twenty-three samples of gemfish (Table 1) were collected by research and commercial trawlers from a total of seven areas off southern Australia (Fig. 1). Of the 763 fish examined, 580 were provided by the Tasmanian Department of Sea Fisheries and 183 by the New South Wales Department of Agriculture, Fisheries Division. Each sample came from a different haul. The viscera of the fish were removed, individually frozen in polyethylene bags with a label giving fish length to caudal fork (LCF) and haul number, air-freighted to Brisbane, and stored frozen. For dissection, the viscera were thawed and the gallbladder fluid searched using a compound microscope (×400) for a maximum of 5 min to determine the presence or absence of Myxozoa. The remaining viscera were thoroughly searched for helminths by dissection and by examining the tissues squashed between glass plates using a dissecting microscope equipped with transmitted light. Helminths in the lumen of the intestine were not counted because of autolysis and because other studies suggest that they may not be good long-term markers for fish (see Lester et al. 1985). In a few cases, the viscera were damaged or incomplete, so the counts of some parasites are from a smaller sample size than that given in the table (see Lester and Sewell 1995).

The term "prevalence" is used to indicate the number of hosts infected divided by the number of hosts examined, and "abundance" is used to indicate the mean number of parasites per host examined in a sample (this includes uninfected hosts) (Margolis et al. 1982).

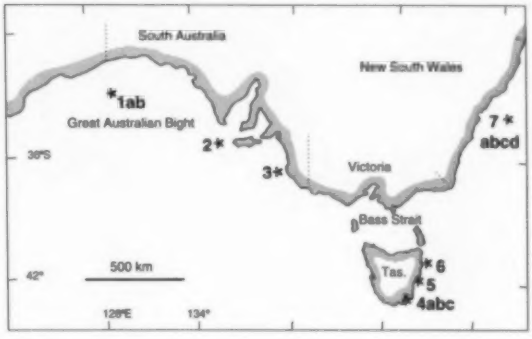
Summary statistics (abundance and prevalence) were calculated for ten parasite taxa and only prevalences for *Ceratomyxa* sp. The distributions of nine parasites selected as long-term markers were analysed together and in combinations with canonical multivariate analysis (Lester et al. 1985, 1988) to relate the parasite fauna between the seven sample areas. Gemfish samples of less than 15 fish were combined with other samples from the same area (Table 1).

For canonical multivariate analyses, parasite numbers were transformed using $\log_e$(parasite number + 1) to stabilise the variance and to bring the overdispersed frequency distribution closer to normal. The transformed values for each parasite species were adjusted to that of a fish with a LCF equal to the mean LCF of all the fish used in the analysis (62 cm), according to a constant calculated from a regression of $\log_e$(parasite number + 1) on fish length. Uninfected fish were excluded from the regression. This procedure minimized bias introduced as a result of the fish samples having different mean lengths.

A random number between −0.05 and +0.05 was added to all parasite data to allow matrix inversion in the canonical multivariate analysis that was otherwise impossible, as some parasite species were absent from some samples. Results of the canonical analyses are displayed as plots of the canonical means for each sample on the first, second, and third canonical axes. Approximate 95 and 99% (not shown) confidence limits around each point were calculated as circles with radii equal to the square root of 5.99/number of fish in sample and the square root of 9.21/number of fish in sample, respectively (Mardia et al. 1979).

If gemfish migrate to and from spawning localities, then the parasite fauna of fish taken during the spawning season, which are a mixture of nonresident and resident fish, may differ from that of fish from the same locality taken outside the spawning season. To maximize our chances of detecting differences, we tested statistically the hypothesis that the numbers of parasites found in adult gemfish taken close to the spawning season (June–August) did not differ from the numbers found in adult gemfish taken in the same areas outside the spawning season (December–April). Adult gemfish were defined as fish greater than 55 cm LCF (Rowling and Reid 1992). We had sufficient fish from two areas, the Great Australian Bight (22 vs. 127 fish from groups 1*a* and 1*b*) and east Tasmania (71 vs. 62 fish from groups 4*a*, 4*b*, 4*c*, 5, and 6) for the analysis. The seasonal differences in the average $\log_e$(parasite number + 1) of three common parasites, *Hepatoxylon trichiuri* (Holton, 1802), *Nybelinia* sp., and *Anisakis* sp. type 1, between gemfish were compared using an analysis of the variance that remained after each of a series of five linear models were fitted to the data. Model 1 was the total variability about the mean in the 282 fish tested. Unexplained variability was progressively reduced by successive models as follows: model 2 removed simple linear effects of fish length; model 3, average differences between the two areas; model 4, common seasonal differences between areas; and model 5, seasonal differences between areas. Hierarchical and other analyses of variance constructed from the results of these models were used to determine whether there was evidence for the presence of seasonal differences, either as differences common to both areas or differences at a particular area, i.e., the variance that remained after models 4 and 5, respectively, were fitted to the data (Lester et al. 1988).

Fig. 1. Map of southern Australia showing the seven areas (asterisks) from which gemfish samples were taken. Area 1, Great Australian Bight; areas 2 and 3, South Australia; areas 4, 5, and 6, east Tasmania; area 7, New South Wales.



Results

Eleven taxa of parasites were recognized and counted (Table 2). *Ceratomyxa* sp. was present as spores in the gallbladder fluid. Plerocercoids of *Hepatoxylon trichiuri* were encapsulated in the mesentery, while those of *Nybelinia* sp. and degenerate *Nybelinia* sp. were usually found encapsulated in the musculature of the stomach wall. Larval *Anisakis* spp. were common in the mesentery and were frequently encapsulated in the wall of the gut, especially the stomach. Types of *Anisakis* were distinguished using the criteria in Smith and Wootten (1978). Larvae of *Terranova* sp. were found encapsulated in the stomach wall or occasionally the mesentery. The hemiuroid digenean was invariably in a state of degeneration in the mesentery and was usually encapsulated by the host. Easily recognized by its large size (5–10 mm) and prominent ventral sucker, it was identified as probably a sclerodistomid or hirudinellid (Dr. R.A. Bray, The Natural History Museum, London, personal communication). Unidentifiable cysts of similar size were not found, suggesting that the parasite is able to endure in gemfish in a recognizable form for long periods. *Rhadinorhynchus* sp. was found encapsulated in the mesentery and free in the intestinal lumen. Only *Rhadinorhynchus* from the mesentery were counted and used in the analyses. *Corynosoma* sp. was encapsulated in the mesentery. All but the *Ceratomyxa* were immature forms, making species identification speculative. Representative specimens of the nine species of parasites listed in Table 2 are lodged in the Queensland Museum, catalogue numbers QM G211885–211896 inclusive.

Parasites were evaluated for their probable longevity in fish and therefore their reliability as host population markers. *Ceratomyxa* sp. was excluded from the analyses because gall bladders were either not intact or not present

Table 2. Abundance and prevalence (%) of selected parasite species found in the viscera of gemfish from the seven areas shown in Fig. 1 (untransformed data).

Area	1*a*	1*b*	2	3	4*a*	4*b*	4*c*
Average LCF (cm)	55	59	56	69	60	58	63
S[a]	10	15	8	12	8	6	10
R	37–90	38–96	42–73	37–96	35–79	41–83	43–86
No. of fish	139	44	29	71	50	94	44
Ceratomyxa sp.							
P (%)	11	22	15	26	10	28	27
Hepatoxylon trichiuri							
A	0.17	0.23	0.00	0.28	0.28	0.52	0.30
S	0.48	0.71	0.00	0.72	0.61	0.86	0.55
R	0–3	0–4	0	0–4	0–3	0–4	0–2
P (%)	14	14	0	18	22	34	25
Nybelinia sp.							
A	2.29	8.86	0.55	3.10	2.00	0.52	2.23
S	4.21	35.95	1.50	5.32	3.10	1.10	3.82
R	0–37	0–232	0–8	0–26	0–15	0–5	0–17
P (%)	60	66	31	59	56	28	57
Degenerate *Nybelinia* sp.							
A	1.67	9.70	0.86	4.33	nc	0.61	nc
S	3.52	35.18	1.98	8.25	nc	2.00	nc
R	0–25	0–190	0–9	0–38	nc	0–13	nc
P (%)	45	66	29	53	nc	23	nc
Anisakis sp., type 1							
A	2.56	3.20	0.07	1.94	0.48	0.22	1.02
S	13.85	8.72	0.26	5.59	1.61	0.76	1.80
R	0–157	0–50	0–1	0–36	0–10	0–5	0–7
P(%)	36	43	7	43	18	12	41
Anisakis sp., type 2							
A	0.14	0.52	0.28	0.06	0.18	0.13	0.39
S	0.56	2.43	1.16	0.23	0.44	0.49	0.87
R	0–5	0–16	0–6	0–1	0–2	0–3	0–4
P (%)	10	16	7	6	16	9	23
Degenerate *Anisakis* sp.							
A	0.01	0.57	0.00	0.04	0.02	0.01	0.00
S	0.17	3.02	0.00	0.20	0.14	0.10	0.00
R	0–2	0–20	0–0	0–1	0–1	0–1	0–0
P (%)	1	11	0	4	2	1	0
Terranova sp.							
A	0.02	7.68	0.03	0.01	0.12	0.00	0.02
S	0.19	49.72	0.19	0.12	0.39	0.00	0.15
R	0–2	0–330	0–1	0–1	0–2	0–0	0–1
P (%)	1	16	3	1	10	0	2
Hemiuroid sp.							
A	0.23	0.18	0.00	0.07	0.00	0.03	0.00
S	0.52	0.58	0.00	0.26	0.00	0.18	0.00
R	0–2	0–3	0–0	0–1	0–0	0–1	0–0
P (%)	19	11	0	7	0	3	0
Rhadinorhynchus sp.							
A	0.07	0.21	0.03	0.01	0.04	0.09	0.09
S	0.23	0.46	0.19	0.12	0.20	0.32	0.36
R	0–1	0–2	0–1	0–1	0–1	0–2	0–2
P (%)	7	18	3	1	4	7	7
Corynosoma sp.							
A	0.01	0.02	0.03	0.00	0.00	0.01	0.05
S	0.08	0.15	0.19	0.00	0.00	0.10	0.21
R	0–1	0–1	0–1	0–0	0–0	0–1	0–1
P (%)	1	2	3	0	0	1	5

Table 2 (*concluded*).

Area	5	6	7*a*	7*b*	7*c*	7*d*	*r*
Average LCF (cm)	57	50	74	74	69	70	
S	7	7	12	9	9	7	
R	47–78	40–67	53–96	60–95	54–107	55–87	
No. of fish	59	50	29	35	63	56	
Ceratomyxa sp.							
P (%)	9	10	33	14	nc	nc	
Hepatoxylon trichiuri							
A	0.66	0.42	0.41	0.31	0.68	0.43	0.27**
S	0.94	0.86	0.91	0.93	1.27	0.81	
R	0–4	0–5	0–4	0–5	0–8	0–4	
P (%)	44	30	24	17	52	61	
Nybelinia sp.							
A	4.10	1.18	8.66	4.77	2.60	2.09	0.33**
S	24.27	2.65	15.33	6.69	6.80	2.86	
R	0–187	0–15	0–81	0–28	0–46	0–11	
P (%)	46	40	76	69	52	61	
Degenerate *Nybelinia* sp.							
A	0.63	0.34	nc	nc	2.35	1.70	0.45**
S	1.13	1.14	nc	nc	3.90	2.04	
R	0–5	0–6	nc	nc	0–18	0–7	
P (%)	33	14	nc	nc	46	47	
Anisakis sp., type 1							
A	0.25	0.18	20.76	8.37	9.03	2.46	0.55**
S	0.71	0.48	54.32	13.71	54.01	3.61	
R	0–4	0–2	0–269	0–60	0–430	0–18	
P (%)	17	16	79	69	56	63	
Anisakis sp., type 2							
A	0.22	0.14	0.97	0.09	0.33	0.16	0.13
S	0.72	0.50	3.53	0.28	0.93	0.46	
R	0–5	0–3	0–19	0–1	0–4	0–2	
P (%)	15	10	28	9	16	13	
Degenerate *Anisakis* sp.							
A	0.00	0.00	0.07	0.03	0.13	0.00	0.53*
S	0.00	0.00	0.37	0.17	1.01	0.00	
R	0–0	0–0	0–2	0–1	0–8	0–0	
P (%)	0	0	3	3	2	0	
Terranova sp.							
A	0.10	0.04	4.07	0.23	0.06	0.21	0.06
S	0.66	0.20	19.81	0.49	0.40	1.11	
R	0–5	0–1	0–107	0–2	0–3	0–8	
P (%)	3	4	28	20	3	7	
Hemiuroid							
A	0.00	0.02	0.00	0.00	0.00	0.02	0.25
S	0.00	0.14	0.00	0.00	0.00	0.13	
R	0–0	0–1	0–0	0–0	0–0	0–1	
P (%)	0	2	0	0	0	2	
Rhadinorhynchus sp.							
A	0.12	0.08	0.79	0.31	0.24	0.16	0.60**
S	0.42	0.34	1.57	1.1	1.04	0.46	
R	0–2	0–2	0–6	0–6	0–8	0–2	
P (%)	8	6	28	11	13	13	
Corynosoma sp.							
A	0.02	0.00	0.24	0.00	0.00	0.00	0.08
S	0.13	0.00	0.95	0.00	0.00	0.00	
R	0–1	0–0	0–5	0–0	0–0	0–0	
P (%)	2	0	0	0	0	0	

Note: Overall correlation coefficient (r) of $\log_e$(parasite number + 1) to fish caudal fork length (LCF) excludes counts where parasite number equals zero; *, 95% probability, **, 99% probability. For raw data see Lester and Sewell (1995).

[a]A, abundance; S, standard deviation; R, range; P, prevalence; nc, not counted in any fish from this area.

in all samples and because related species are believed to be short lived in fish (Lester et al. 1988). Degenerate *Nybelinia* sp. (possibly degenerate forms of intact *Nybelinia* sp.) were small (approx. 0.5–1 mm) compared with *Nybelinia* sp. (approx. 2–3 mm). Their small size and state of degeneration made identification and accurate counts difficult and time consuming. Significant errors were therefore likely to exist in counts of this parasite and it was also excluded from the analyses.

Nine taxa of parasites were considered suitable for use: the larval cestodes H. trichiuri and *Nybelinia* sp.; the larval anisakid nematodes *Anisakis* sp. type 1, *Anisakis* sp. type 2, *Terranova* sp., and degenerate *Anisakis* sp.; the digenean hemiuroid sp.; and the acanthocephalans *Rhadinorhynchus* sp. and *Corynosoma* sp. The *Anisakis* type 1 corresponded to the *A. simplex* larva of Hurst (1984); the *Anisakis* type 2 may be *Anisakis physeteris* Baylis, 1923. Six of the parasites were likely to be long lived in gemfish, as they showed a positive correlation with fish LCF (Table 2). Information on the longevity of parasites related to these and the other three selected parasites in other fish species suggested that all nine were likely to remain recognizable in the fish for an extended time and thus make good stock markers.

Canonical multivariate analysis of data from the nine selected parasite taxa showed that the parasite faunas from areas 2, 3, 4, 5, and 6 (South Australia and east Tasmania) were similar (Figs. 2 and 3). Groups 4*a*, 4*b*, and 4*c*, all from the same area (east Tasmania) but taken in different years, fell together. Group 3 (South Australia) fell close to all five east coast groups and overlapped with three of them, indicating that the parasite faunas showed strong similarities.

The samples from the Great Australian Bight fell together at one side of the figure (Figs. 2 and 3; groups 1*a* and 1*b*). These two samples were taken from the same location but in different seasons and years (Table 1). Both Bight samples fall close to one of the South Australian samples (group 3) and group 1*b* overlaps with two of the New South Wales samples (groups 7*b* and 7*d*), which suggests either some mixing or a common enzootic parasite fauna. Nevertheless, gemfish from the Great Australian Bight (groups 1*a* and 1*b*) are distinct from those of South Australia (groups 2 and 3) and Tasmania (groups 4*a*, 4*b*, 4*c*, 5, and 6).

The parasite fauna of fish taken off New South Wales at the end of the spawning season (group 7*a*) is different from that of most other groups (Figs. 2 and 3), suggesting that there could be change in the composition of spawning fish between the start and the end of the spawning season.

The first two canonical axes accounted for 67% of the variation. The third axis accounted for a further 11%. To determine the parasite with the greatest discriminating power between areas, we ran a simple ANOVA for each parasite and looked for the parasite that had the most significant difference between areas (the greatest F and the smallest p).

Anisakis sp. type 1 discriminated best, followed by the hemiuroid, *Nybelinia*, *H. trichiuri*, and *Terranova* sp. *Anisakis* type 2 had the least discriminating power.

To look for evidence of seasonal movement of mature fish, we compared the parasite fauna of large fish caught within the spawning season to that of fish caught in the same area outside the spawning season, for two areas, the Great Australian Bight and east Tasmania. We found that there were significantly different numbers of *H. trichiuri* (df 1/93; $F = 4.38$; $p < 0.5$), *Nybelinia* sp. (df 1/91; $F = 6.43$; $p < 0.05$), and *Anisakis* type 1 (df 1/91; $F = 18.94$; $p < 0.01$) in both areas, indicating that the composition of adult fish caught at these sites during the spawning season was different from that caught there outside the spawning season.

Discussion

Correct assessment of parasite longevity is crucial to the successful interpretation of parasitological data in terms of fish stock structure (Lester 1990). A valuable indication can be the correlation between parasite number and host length (i.e., age). Parasites that are long lived and that infect fish regularly over a long period accumulate in fish and their numbers show a positive correlation with host age (Shotter 1972; Lester et al. 1985). A negative correlation suggests that the parasite is being lost from fish over time and therefore is short lived in the fish and not a useful population indicator. This could be an erroneous conclusion if there is no indication of parasite death, such as the occurrence of degenerate forms. In such a case, a negative correlation would occur if infected fish were lost from the population more rapidly than uninfected ones, or if older fish were exposed to fewer parasites when they were juveniles than younger fish. However, in general, a long lived parasite should show a positive correlation with fish length, as did most of the parasites used in the present study.

Rowling (1987) suggested that gemfish in the management area for the South East Trawl Fishery (the east coast of Australia from Sydney to Tasmania) should be considered as a single stock reliant upon a single spawning area off the northern coast of New South Wales. Our results from the multivariate analysis of parasite fauna support this suggestion. Furthermore, contrary to current hypotheses regarding stock structure, we demonstrate that there is much similarity between fish off South Australia and the east coast. The parasitological evidence suggests that fish on the eastern and western sides of Bass Strait belong to a single stock. Possibly after the NSW spawning run, fish disperse as far as South Australia. Fish in the Great Australian Bight appeared to belong to a different stock.

In New Zealand, seasonal catch data suggest that the gemfish fishery comprises at least two stocks: a large southern west coast stock and a smaller northern east coast stock. The fish are believed to migrate north in autumn to spawn (Hurst 1988).

Our data indicate that gemfish within a single fishing area can have different histories. The parasite fauna of fish from New South Wales taken towards the end of the

Fig. 2. Canonical multivariate analysis for 13 samples of gemfish (Table 1) from the seven areas shown in Fig. 1. The positions of the sample means on the first and second canonical axes are plotted and their 95% confidence limits shown.

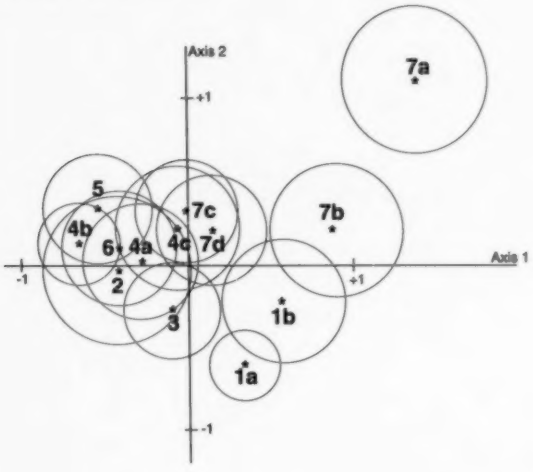
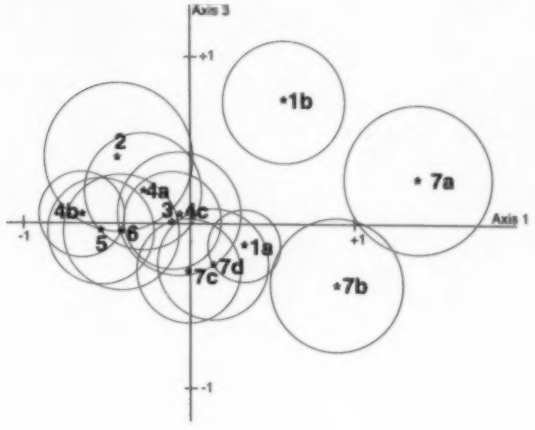


Fig. 3. Plots of the first and third canonical axes of the samples shown in Fig. 2, together with 95% confidence limits.



spawning season (group 7*a*) was significantly different from that of all other east coast and Tasmanian samples. Group 7*a* consisted of many large fish, so a length associated phenomenon could be the cause of the separation, although length was accounted for in the canonical analysis. Our separate analysis on only large fish showed that fish caught at two localities outside the spawning season differed from those caught there during the spawning season. The two analyses taken together suggest that the spawning run off New South Wales contains gemfish resident at other times of the year in localities not sampled in the present study. The fish may have been residents of the New South Wales coast, which were later diluted by southern fish in their prespawning run, or they may be a component that moves in from offshore. If there is a resident component of the fishery, rather than a random dispersion following spawning, one would expect large resident fish to maintain a parasite fauna distinct from those fish that move further south. We had insufficient data to test this hypothesis but our results suggest that gemfish from New South Wales do not disperse randomly after spawning.

Acknowledgements

We thank the Tasmanian Department of Sea Fisheries and the Fisheries Division, New South Wales, for gemfish samples. Dr. I. Beveridge (Melbourne University, Victoria) and Dr. R.A. Bray (The Natural History Museum, London, UK) provided expert help with parasite taxonomy. Dr. G.N. Berry, Dr. T.H. Cribb, Dr. B. Heath, Ms. J.M. Masel, and Dr. G.A. West, University of Queensland (UQ) helped with dissections. Mr. Tony Barnes (UQ) provided statistical advice and Mr. Steven Heng (UQ) assisted with computer programming. Mr. K. Rowling (Fisheries Division, New South Wales), Dr. J.R. Arthur (Maurice Lamontagne Institute, Mont-Joli, Que.), and an anonymous reviewer provided valuable critiques of an earlier draft of the manuscript. Mr. Phil Wharton used computer-aided drawing to provide final copies of the illustrations. The project was funded by a grant from the Fishing Industry Research Trust Account (FIRTA 84/27) to R.J.G.L.

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Influence of the transmission of parasites from prey fishes on the composition of the parasite community of a predatory fish

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Abstract: Helminth parasites and diet of seven freshwater fishes (*Lota lota* and six common prey species) from the Bothnian Bay, Baltic Sea, were studied monthly or bimonthly during 1978. Twenty-one of the 32 parasites with complex life cycles were shared between *Lota lota* and its prey fishes and are thus transmissible from prey to predator. *Gymnocephalus cernuus* and *L. lota* had the greatest number of shared species (13). Larval and adult cestodes, nematodes, and acanthocephalans could re-establish in the predator, but only one adult trematode was capable of this transition. Infracommunity species diversity was highest in *L. lota* ($e^{H'}$ = 3.54), which also had the most species (24), the highest mean number of species and individuals of a given species per fish (6.3 and 62, respectively), and the greatest number of worms in one fish (520). Variety of diet was key in determining exposure to parasite species. However, most specificity finally determined if a given parasite could establish and mature. No ecologically explicable suites of parasites were found in any fish species, except in a few cases where parasites used related intermediate hosts. However, the composition of these suites was not retained in the predator. Unlike in *L. lota*, important parasites of prey fishes were typically specialists.

Résumé : Nous avons étudié à un rythme mensuel ou bimensuel, pendant l'année 1978, les helminthes parasites et l'alimentation de sept poissons d'eau douce (*Lota lota* et six espèces proies communes) du golfe de Botnie, en mer Baltique. Vingt-et-un des 32 parasites ayant des cycles de vie complexes se retrouvaient chez *Lota lota* et ses poissons proies, et sont donc transmissibles de la proie au prédateur. Ce sont *Gymnocephalus cernuus* et *L. lota* qui avaient en commun le plus grand nombre d'espèces (13). Les larves et les adultes de cestodes, de nématodes et d'acanthocéphales pouvaient se réétablir chez le prédateur, mais un seul trématode adulte était capable d'accomplir ce transfert. Au niveau infracommunautaire, la diversité spécifique était la plus haute chez *L. lota* ($e^{H'}$ = 3,54), qui comptait aussi le plus grand nombre d'espèces (24), le plus grand nombre moyen d'espèces et d'individus d'une espèce donnée par poisson (respectivement 6,3 et 62), et le plus grand nombre de vers dans un seul poisson (520). La variété de l'alimentation jouait un rôle clé dans l'exposition aux espèces parasites. Toutefois, c'est la spécificité par rapport à l'hôte qui déterminait en fin de compte si un parasite donné pouvait s'établir chez l'hôte et y atteindre la maturité. Nous n'avons trouvé chez aucune espèce de poisson une suite de parasites écologiquement explicable, sauf dans quelques cas où les parasites utilisant des hôtes intermédiaires apparentés. Toutefois, la composition de ces suites ne se retrouvait pas chez le prédateur. À la différence de ce qu'on observait chez *L. lota*, les parasites importants des poissons proies étaient typiquement des spécialistes.

Introduction

Component communities of parasites of freshwater fishes are determined, among other things, by the degree to which the parasite species are exchanged between different species of hosts. Exchange is most common between related and abundant host species (Leong and Holmes 1981). The possibility of exchange, however, is dependent on several factors, of which the feeding habits and variety of diet of the host, and the specificity of the parasite species are most important (Leong and Holmes 1981; Kennedy et al. 1986; Janovy et al. 1992). Exchange of intestinal helminths is well documented in water birds (Neraasen and Holmes 1975; Bush and Holmes 1986). Among four species of grebes (Podicipedidae) the degree of overlap in the feeding habits of co-occurring hosts contributed to the sharing of helminths between hosts, and this was especially true in cases where the hosts were related species (Stock and Holmes 1987). Ducks acquire intestinal helminths by eating

Received March 31, 1994. Accepted February 8, 1995.
J12797

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Fig. 1. Map of the Baltic Sea showing the location of the study area.

infected intermediate hosts, typically invertebrates (Bush and Holmes 1986). Cestodes of ducks are often very small, site specific, and much more abundant than cestodes in fish, and several species of cestode can co-occur in the same individual bird (Bush and Holmes 1986; Kennedy et al. 1986). In fishes, adult cestodes tend to be large, less site specific, and very host specific, as shown for those species occuring in the fishes of the Bothnian Bay, Baltic Sea (Andersen and Valtonen 1990). In fish communities, cestode infections of unsuitable hosts, such as paratenic or transport hosts, may sometimes be important for transmission to required final hosts (Andersen and Valtonen 1990); this is not the case in birds.

We were able to study the metazoan parasites and stomach contents of 31 common fish species collected on a seasonal basis during 1978 from the Bothnian Bay (see Valtonen 1983*a*; Valtonen and Crompton 1990; Andersen and Valtonen 1990, 1992). From this material, we analyzed the diet of adult burbot (*Lota lota*) and of other six fish species, which comprised 80% of the fish specimens identified in its diet. The data from monthly or bimonthly samples of fish collected in 1978 were pooled to reduce trends in the seasonal variation of many parasite species.

We wanted to explain the role played by the transmission of parasites from the six prey fishes to *L. lota* in structuring the parasite community of the predator. We also wanted to determine whether the helminth parasites in the infracommunities of the prey fishes and the predator form any groups or suites that tend to co-occur, as demonstrated for the intestinal helminths of lesser scaup ducks in Canada by Bush and Holmes (1986). If such groups do occur in prey fishes, they might also be transmitted to *Lota lota*. In this study, we suggest that the transmission of cestodes and other helminths, both adults and larvae, from a prey fish to a predator is an important factor that affects the structure of the parasite community of the predator even if the prey fishes are not species related to the predator. Also, the importance of specificity of a parasite species in successful transmission to a new host is demonstrated.

Materials and methods

The Bothnian Bay is a unique aquatic environment in the northern part of the Gulf of Bothnia. The study area (Fig. 1) in the northeastern part of the Bothnian Bay is in the most oligotrophic and least saline (3‰) region of the Baltic Sea and is ice covered for about 6 months of every year. Its fish fauna includes 30 reproducing species, most of which are of freshwater origin. Marine species also occur, but few of them are able to breed (Andreasson and Petersson 1982). A comprehensive study on the parasites of the most common fishes in the northeastern part of the Bothnian Bay was conducted between 1975 and 1983. The data were collected on a seasonal basis and the sample size of fish varied between 20 and 30 individuals/species in most cases (see Valtonen 1983*a*; Valtonen et al. 1988; Valtonen and Crompton 1990; Andersen and Valtonen 1990, 1992). In this study we deal with the data collected between January and December of 1978 (Table 1). It is essential to note that a similar number of each fish species was collected throughout the year in most cases. For example, the samples of *Osmerus eperlanus* were collected every month (32, 31, 35, 30, 22, 20, 25, 30, 30, 22, 30, and 25 fish from January to December, respectively). The stomach contents of *Lota lota* were analyzed and the six most common fish prey species were chosen for parasitological analysis. As a result, a total of 1520 fishes was studied. Parasites of those four fish species that were found more incidentally in the stomachs of *L. lota* were also considered. Fishes were caught by local commercial fishermen using trawl and, occasionally, fyke nets at monthly or sometimes bimonthly intervals (Table 1). During winter, samples were obtained by the use of seine nets under the ice.

Parasitic crustaceans, annelids, and glochidia larvae of freshwater bivalves were studied from the gills of the fishes. Larvae of cestodes, nematodes, and digeneans from the inner organs and musculature were collected and counted. Eye lenses were examined from about 10 fish per sample. Only cyst-forming myxosporean protozoans from the muscles were studied. Intestinal parasites were counted and identified to species. However, the adult nematodes *Haplonema hamulatum* Moulton, 1931 and *Raphidascaris acus* (Bloch, 1779) were not always separated at the infracommunity level. Parasites were handled and identified using standard parasitological methods.

The abundances of parasite species were aggregated and non-normal in distribution. Therefore, Spearman rank-correlation coefficients were used as a measure of the association of the parasite species in infracommunities. The relationships of these associations are presented in three-dimensional space, where the coordinates of the species were calculated using the multidimensional scaling method. Multidimensional scaling was performed using SYSTAT software (Wilkinson 1990).

The richness and heterogeneity of infracommunities were described by the mean number of species and the Shannon–Wiener diversity index. The Shannon–Wiener index weights rare species more than the Simpson diversity index. The formulation used is $N_1 = e^{H'}$ (Krebs 1989). Indices calculated in this way can be interpreted more

Table 1. Fish studied from the Bothnian Bay, Baltic Sea, during 1978.

	Total no. of fish examined	Length, mm				Sampling period	No. of samples	Mean no. of fish
		Min.	Max.	Mean	SD			
Lota lota	83	310	715	470	92.1	Jan. – Dec.	9	9
Gasterosteus aculeatus	431	31	85	66	6.9	Jan. – Dec.	11	39
Gymnocephalus cernuus	330	54	199	127	20.4	Jan. – Dec.	11	30
Osmerus eperlanus	332	69	259	156	23.0	Jan. – Dec.	12	28
Coregonus albula	164	111	226	166	24.1	Jan. – Dec.	7	23
Myoxocephalus quadricornis	162	120	320	212	46.1	Jan. – Dec.	6	27
Pomatoschistus minutus	18	23	52	38	8.4	April	1	18

clearly in relation to the number of species than the original Shannon–Wiener index.

Similarities between infracommunities of each fish species and also between the predator and its prey fishes were measured using percentage similarity (PS) and Jaccard's dichotomy similarity indices (Krebs 1989). Only fishes in which all parasites had been studied were used for assessing similarities. Twenty-five pairs of fish were selected randomly for each fish species. PS and Jaccard's similarities were calculated for each species pair and mean values determined.

Results

Fish diet

Among the seven fish species studied in the Bothnian Bay, *Lota lota* had the greatest proportion of fish in its diet (Fig. 2), although smelt, *Osmerus eperlanus*, and fourhorn sculpin, *Myoxocephalus quadricornis*, also had fish in their stomachs. Planktonic crustaceans were the most common food items of *O. eperlanus*, threespined stickleback, *Gasterosteus aculeatus*, and vendace, *Coregonus albula*. The occurrence of molluscs in the stomachs of all fishes studied was low. Other crustaceans, including ostracods, *Saduria entomon*, *Gammarus* spp., *Monoporeia* (=*Pontoporeia*) *affinis*, *Asellus aquaticus*, and Mysidae, were also well represented in *L. lota* and ruffe, *Gymnocephalus cernuus*, and to some degree in *O. eperlanus*. Ostracods formed the largest proportion of the diet of sand goby, *Pomatoschistus minutus*. Insect larvae were found most often in the stomachs of *G. cernuus* and *G. aculeatus*. The greatest variety in the food content was found in *G. cernuus* (14 groups of food items; see Fig. 2), *O. eperlanus* and *L. lota* (nine groups), and *G. aculeatus* (eight groups).

The variety of food items in *L. lota* increased when the fish prey consumed were analyzed and included. Eleven fish species were identified, of which *G. cernuus*, *O. eperlanus*, *G. aculeatus*, *P. minutus*, *C. albula*, and *M. quadricornis* were the most common, comprising 80% of all identified food objects (Table 2). Fish identified in the stomach contents of *O. eperlanus* and *M. quadricornis* consisted mainly of *P. minutus*, 77 and 73% of all identified fish, respectively. Other fish species consumed by the *O. eperlanus* were *O. eperlanus*, *Pungitius pungitius* (tenspined stickleback), *Clupea harengus* (Atlantic herring), and *G. aculeatus*, while *M. quadricornis* consumed *C. albula* and *G. aculeatus*.

Parasites

One protozoan and 38 metazoan parasite species were found in the seven fish species studied (Table 3). *Lota lota* had the greatest number of parasite species (24), followed by *G. cernuus* and *G. aculeatus* (15 each), *O. eperlanus* (11), *M. quadricornis* (10), *C. albula* (9), and *P. minutus* (7) (Table 3). Cestodes were the most numerous helminth group (12 species), followed by nematodes (8), acanthocephalans (7), and trematodes (5).

Of the 32 helminth species found, 11 were allogenic and 21 autogenic species (Table 3). Autogenic species are those in which the entire life cycle is completed within an aquatic ecosystem, while allogenic species mature in birds or mammals (see Esch et al. 1988).

Parasite species were divided into important, secondary, and rare species. Our division is similar to the "core – satellite" system (see Stock and Holmes 1987), but because co-occurences of the species are not emphasized, we prefer to include more of the more common species as "important." Species less common are termed "secondary" species, while occasional species are termed "rare." Our categorization is based on prevalence as seen in Fig. 3, but also on mean intensities (no. of worms/no. of infected fish), because statistically significant positive correlations between prevalence and mean intensity were found, for example, in the case of *L. lota* (Spearman correlation coefficient $r_s = 0.44$, $p = 0.043$) (Fig. 4). In Table 4, the mean abundances of parasite species are given in those cases where they are ≥1. As shown in Fig. 3, we have chosen a prevalence of 40% as the lower limit for important species, except for *M. quadricornis*, where the limit is 20%. Parasite species with prevalence of less than 10% have been recorded as rare species in all fish species except for *G. cernuus*, *G. aculeatus*, and *M. quadricornis*, where the limit is 5%. In *L. lota*, 33% of parasite species were rare species. In this data, important species can be distinguished more clearly than secondary and rare species. No clear bimodal or trimodal distribution of parasite species relative to prevalence was found. This may be due to the depauperate parasite communities of the present freshwater fishes.

Fig. 2. Groups and proportions of food items in the stomach contents of seven fish species in the Bothnian Bay, Baltic Sea.

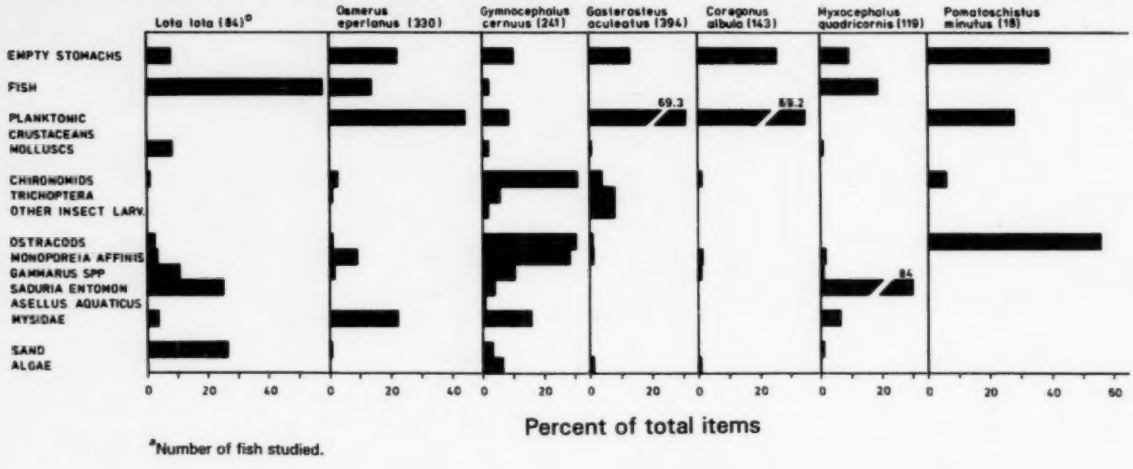


Table 2. Proportion (%) of different fish species present in the diet of *Lota lota* ($n = 56$) in the Bothnian Bay, Baltic Sea.

Species	Proportion (%)
Gymnocephalus cernuus	23.2
Osmerus eperlanus	14.3
Gasterosteus aculeatus	12.5
Pomatoschistus minutus	12.5
Coregonus albula	10.7
Myoxocephalus quadricornis	7.1
Rutilus rutilus	5.4
Coregonus lavaretus	5.4
Salmo trutta	3.6
Pungitius pungitius	3.6
Lota lota	1.8
Total	100

Figure 5 gives in descending order the prevalences of all parasites found. We have depicted the cut-off points where the important, secondary, and rare species of each fish species have been separated. Furthermore, we indicate the parasite species that are not transmitted to a predator via ingestion, i.e., ectoparasites with direct life cycles and *Diplostomum spathaceum* (Rudolphi, 1819) and *Ichthyocotylurus* spp., whose cercariae penetrate fish directly. *Henneguya zschokkei* (Gurley, 1894) and the six helminth species that occur in some prey fishes but were never encountered in *L. lota* are also indicated. *Diplostomum spathaceum* was one of the important species of all the fish species except for *P. minutus* (Fig. 5, Table 3). The number of important species was highest (9) in *L. lota* and varied from one to four in other fish species. The number of rare species was seven in *L. lota*, *G. cernuus*, and *G. aculeatus*, five in *O. eperlanus* and *M. quadricornis*, and four and three, respectively, in *C. albula* and *P. minutus*.

When the prevalences of parasites in *L. lota* and its prey fishes are compared, four groups can be distinguished. (*i*) Parasites with a distinctly higher prevalence in *L. lota* than in its prey fishes. These include two cestode plerocercoids (*Triaenophorus nodulosus* (Pallas, 1781) and *Diphyllobothrium latum* (Linnaeus, 1758)), one adult cestode (*Eubothrium rugosum* (Batch, 1786)), and two nematodes (*Raphidascaris acus* and *Haplonema hamulatum*) (see Fig. 5). (*ii*) Species that have lower prevalences in *L. lota*. These include *Proteocephalus* spp., *Diphyllobothrium ditremum* (Creplin, 1825), and *Bunodera luciopercae* (O.F. Müller, 1776). (*iii*) Parasites whose prevalence was similar in *L. lota* and in at least some prey fish. This group was the greatest in *L. lota* (see Fig. 5). (*iv*) Species that were found only in *L. lota*. In addition to three ectoparasites, three helminths, *Haplonema hamulatum*, *Pseudocapillaria salvelini* (Polyansky, 1952), and *Acanthocephalus lucii* (O.F. Müller, 1776), were not found in any of the six prey fish species (see Fig. 5).

Lota lota consumed more rarely roach (*Rutilus rutilus*), whitefish (*Coregonus lavaretus*), brown trout (*Salmo trutta*), tenspined stickleback, (*Pungitius pungitius*), and *L. lota* (Table 2). Each of these fish species harboured more or less specific parasites that were not found in *L. lota*, such as *Schistocephalus* sp. in *P. pungitius*; *Triaenophorus crassus* Forel, 1868, *Phyllodistomum umblae* (Fabricius, 1780), and *Cystidicola farionis* Fischer, 1798 in *C. lavaretus*; *Caryophyllaeus fennica* (Schneider, 1902) in *R. rutilus*; and *Eubothrium crassum* (Bloch, 1779) in *S. trutta*. However, even these rare food items may be sources of some parasite species to the predator. For example, *D. dendriticum* (Nitzsch, 1824) is common (prevalence of 75%) in *S. trutta* and also occurs in *C. lavaretus* (1.4%); *Triaenophorus nodulosus* (2.1%) and Eubothrium sp. (1.3%) also occur in *P. pungitius*; larvae of *Raphidascaris acus* are common in *R. rutilus* (10.8%) and also occur in *C. lavaretus* (3.8%). Acanthocephalans are also often shared between *L. lota* and these four occasional food items. *Neoechinorhynchus rutili* (O.F. Müller, 1780) may be acquired from all these

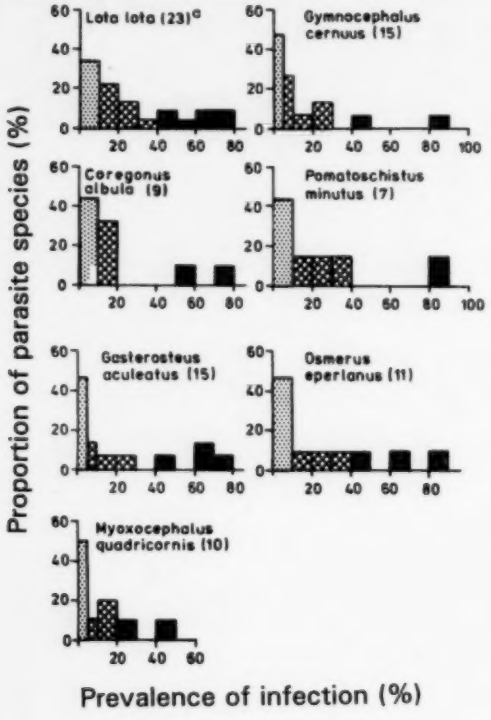
Table 3. Parasite species found in the fish of the Bothnian Bay, Baltic Sea, during 1978.

	L. lota	*G. aculeatus*	*G. cernuus*	*O. eperlanus*	*M. quadricornis*	*C. albula*	*P. minutus*
Protozoa							
Henneguya zschokkei						C	
Cestoda							
**Diphyllobothrium dendriticum* (p)	B	B			C		
**D. ditremum* (p)	C	A		C		B	
**D. latum* (p)	A		C				
Eubothrium rugosum	A						
Eubothrium spp.		C	C				
Proteocephalus cernuae			A				
P. exiguus						A	
P. filicollis		C					
P. longicollis				B			
Proteocephalus sp.	B				B		A
**Schistocephalus solidus* (p)		A					
Triaenophorus crassus (p)						B	
T. nodulosus (p)	A	B	B	C			
Trematoda							
Bunodera lucioperca	C		B				B
**Diplostomum spathaceum* (m)	A	A	A	A	A	A	B
**Ichthyocotylurus erraticus* (m)				C		B	
**I. variegatus* (m)			A				
Sphaerostoma sp.							C
Nematoda							
Camallanus lacustris	B		B		C		C
**Contracaecum osculatum* (l)	B			C	B		
Cystidicola farionis				A		C	
Desmidocercella sp.		C					
**Eustrongylides* sp. (l)	A	C	A		C		
Haplonema hamulatum	A						
Pseudocapillaria salvelini	C						
Raphidascaris acus (l)[a]	A	B	C		C	C	C
Acanthocephala							
Acanthocephalus lucii	C						
**Corynosoma semerme* (j)	B		C	B	A		
**C. strumosum* (j)	B		C	C	B		
Echinorhynchus borealis	C	C	C				
E. bothniensis	A			A	C		
E. salmonis	B		C	B		C	
Neoechinorhynchus rutili	B	B	B				B
Annelida							
Cystobranchus mammilatus	A						
Piscicola geometra	C	C					
Mollusca							
glochidia larvae	C						
Arthropoda							
Argulus foliaceus		C					
Thersitina gasterostei		A					

Note: The occurence of each parasite species in each fish species is given. The status of the parasite species is also indicated: important, secondary, and rare species are indicated by A, B, and C, respectively, and allogenic species by *. j, juvenile; l, larva; m, metacercaria; p, plerocercoid.

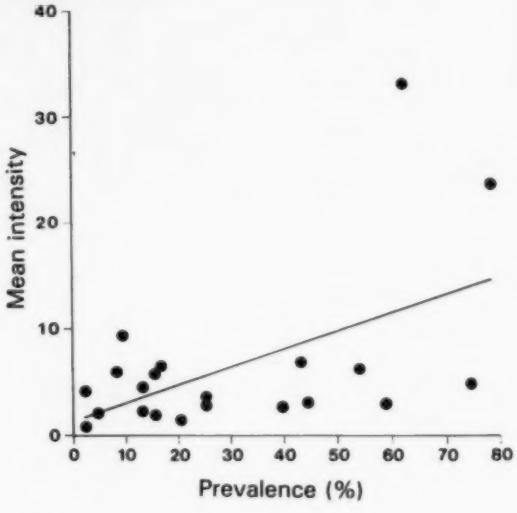
[a]Matures in *Lota lota*.

Fig. 3. Frequency distributions of parasite prevalences for seven fish species from the Bothnian Bay, Baltic Sea. Solid bars, important species; cross-hatched bars, secondary species; stippled bars, rare species.



[a]Number of parasite species.

Fig. 4. The relationship between mean intensity and prevalence of parasite species in *Lota lota* of the Bothnian Bay, Baltic Sea.



food items (max. prevalence of 8.2% in *P. pungitius*), while *Echinorhynchus salmonis* (O.F. Müller, 1780) may be transferred from *S. trutta* and *C. lavaretus* (prevalences of 27 and 44%, respectively). *Echinorhynchus bothniensis* Zdzitowiecki and Valtonen, 1987 is common in *S. trutta* (14.5%) and also occurs occasionally in *C. lavaretus*, as does *E. borealis* Linstow, 1901. Metacercariae of *Diplostomum spathaceum*, which are not transmitted from prey to predator, were also common in all these fish (max. prevalence of 99% in *R. rutilus*).

The number of parasite species per fish was highest in *L. lota*; in most cases three to eight species were found per fish, the maximum being 12 and the mean 6.3 (Fig. 6, Table 5). The lowest mean number of parasite species per fish occurred in *M. quadricornis* (0.9), where the number of fish devoid of parasites was also the greatest (Fig. 6). In *G. aculeatus*, *O. eperlanus*, and *G. cernuus*, the distribution of the number of parasite species per fish was very similar, although the mean number was highest (3) in *G. aculeatus*. The mean abundance was highest in *L. lota* (61.9) and lowest in *M. quadricornis* (5.9) (Table 5).

The diversity and similarity values of the parasite infracommunities in those cases where all of the parasites were studied are given in Table 5. Diversity index is highest in *L. lota* (3.5) and lowest (0.8) in *M. quadricornis*. Percentage similarities do not vary greatly between *G. aculeatus*, *G. cernuus*, and *C. albula* (0.35–0.41). The highest value (0.68) occurs in *O. eperlanus*, although this is not shown as clearly using Jaccard's similarity index. Jaccard's similarity values were quite similar, being slightly over 0.3 in all other fish species except for *M. quadricornis*, where it was 0.05. Percentage similarities between *L. lota* and its prey fishes were always low. The highest value (0.06) occurred between *G. cernuus* and *L. lota*, and the lowest (0.0) between *C. albula* and *L. lota*. The same was true for Jaccard's similarity measures (range of 0.01–0.06).

Some positive interspecific associations between parasites were found in most fish species when the Spearman rank correlation coefficients were presented in three-dimensional space (Fig. 7). In these analyses, only those parasite species that are transmitted via the food web were used. No associations were found in *C. albula*. Among the positively associated groups, no general similarities were found between different fish species using three-dimensional scaling. For instance, *Diphyllobothrium ditremum* and *D. dendriticum* formed a group in *G. aculeatus*. The same was also true for *Eubothrium* juveniles and the larval nematode *Eustrongylides* sp. in *G. aculeatus* (Fig. 7), but this group was not formed in *G. cernuus*, although both species were present. The two positively associated groups in *G. cernuus* were *Echinorhynchus borealis – Triaenophorus nodulosus* and *Camallanus lacustris* (Zoega in O.F. Müller, 1776) – *Raphidascaris acus*. The four species forming a group in *O. eperlanus* were *Echinorhynchus bothniensis*, *E. salmonis*, *Cystidicola farionis*, and *Corynosoma semerme* (Forssell, 1904) (Fig. 7). In *L. lota*, the finding of groups exhibiting some sort of association is more difficult. However, we have indicated four groups in Fig. 7 that are

Table 4. The abundances of metazoan parasites in the seven fish species studied in the Bothnian Bay during 1978 in cases where the abundance was ≥1.

	L. lota	*G. aculeatus*	*G. cernuus*	*O. eperlanus*	*M. quadricornis*	*C. albula*	*P. minutus*
Cestoda							
Diphyllobothrium ditremum		1.5					
D. latum	1.4						
Eubothrium rugosum	3.6						
Proteocephalus cernuae			1.8				
P. exiguus						12.2	
P. longicollis				1.8			
Proteocephalus sp.							22.3
Triaenophorus nodulosus	1.8						
Trematoda							
Diplostomum spathaceum	18.7	5.8	6.8	1.2	4.0	1.4	2.4
Ichthyocotylurus variegatus			3.1				
Nematoda							
Cystidicola farionis				18.1			
Eustrongylides sp.	1.1						
Raphidascaris acus	20.8						
Acanthocephala							
Corynosoma semerme					4.6		
Echinorhynchus bothniensis	3.0			2.1			
E. salmonis	1.1						
Neoechinorhynchus rutili							1.4
Arthropoda							
Thersitina gasterostei		7.9					

totally different from any other group in any prey fish species.

Patterns of possible transfer of parasites between the predator and its six common prey fishes are illustrated in Fig. 8. *Gymnocephalus cernuus* and *L. lota* share the greatest number of species (13 of 15). Nine of 15 species may be transmitted from *G. aculeatus* and 9 of 10 from *M. quadricornis*. Of the 11 species found in *O. eperlanus*, 8 are shared with *L. lota*, as are 5 and 4 from the 7 and 10 species in *P. minutus* and *C. albula*, respectively.

Among the shared helminths, both larval and adult stages of cestodes, nematodes, and acanthocephalans are present, but only one adult trematode, *Bunodera luciopercae* (Table 3). Similarly, both important, secondary, and rare species are shared. When all 11 possible prey fish species are included, six of the nine important species of *L. lota*, for example, are shared with one to as many as all 11 prey fishes (Table 2). Among the species that may be transmitted via the food web, *Raphidascaris acus* may be acquired from a maximum of eight prey fishes, in five of which it occurs as a rare species. Excluding *D. spathaceum*, only two important species of *L. lota* were also important species of prey fishes (*Echinorhynchus bothniensis* in *O. eperlanus* and *Eustrongylides* sp. in *G. cernuus*).

Discussion

Janovy et al. (1992) noted that the presence of a parasite in a host is proof that the chemical and physical environment provided by the host is adequate for the parasite, at least temporarily, and that the parasite is a manifestation of the evolutionary history of both the host and the parasite. The same authors also agree with Brooks and McLennan (1991) that this is not necessarily a product of co-evolution. Janovy et al. (1992) have also proposed that the proximal factors that control host–parasite encounters are largely ecological.

The results of this study show that the major structuring factors involved in defining the component community structure of a predatory fish species in the Bothnian Bay are associated primarily with the specificity of the parasites and the accumulation of the helminths by the predator through the food chain. According to Stock and Holmes (1987), most of the core species of grebes are specific to their hosts. This is also true for the prey fishes in the Bothnian Bay. The only generalist important species in their parasite communities are the eye fluke *Diplostomum spathaceum* and the juvenile acanthocephalan *Corynosoma semerme* in *Myoxocephalus quadricornis*. The situation is totally different in *Lota lota*. Of the nine important species only three (*Cystobranchus mammillatus* (Malm, 1863), *Haplonema hamulatum*, and *Eubothrium rugosum*) are specialists and occur only in *L. lota*. Thus many common and abundant important species of the prey fishes are unable to re-establish as important species in *L. lota*. We suggest that the reason for this is the specificity of the parasites.

Another important factor in structuring the parasite component communities of our fishes is the spectrum of the diet, which determines the amount of exposure to invading parasites. The variety of food items is widest in the predatory *L. lota* (8 invertebrate groups and 11 prey fish species).

Fig. 5. Prevalences of parasites in descending order. The cut-off points between important and secondary species and between secondary and rare species are indicated by arrows. Cross-hatched bars, parasites having distinctly higher prevalences in *Lota lota* than in its prey fishes; stippled bars, parasites having lower prevalences in *L. lota*; diagonal hatched bars, parasites exhibiting no essential change in prevalence between *L. lota* and at least some prey fishes; open bars, parasites found only in *L. lota*; solid bars, parasites not transmitted to a predator via ingestion. Parasite names are abbreviated as follows: first letter of the generic name together with first four letters of the species name. If the species is given as spp. or sp. then first three to four letters of the generic name, respectively, have been used.

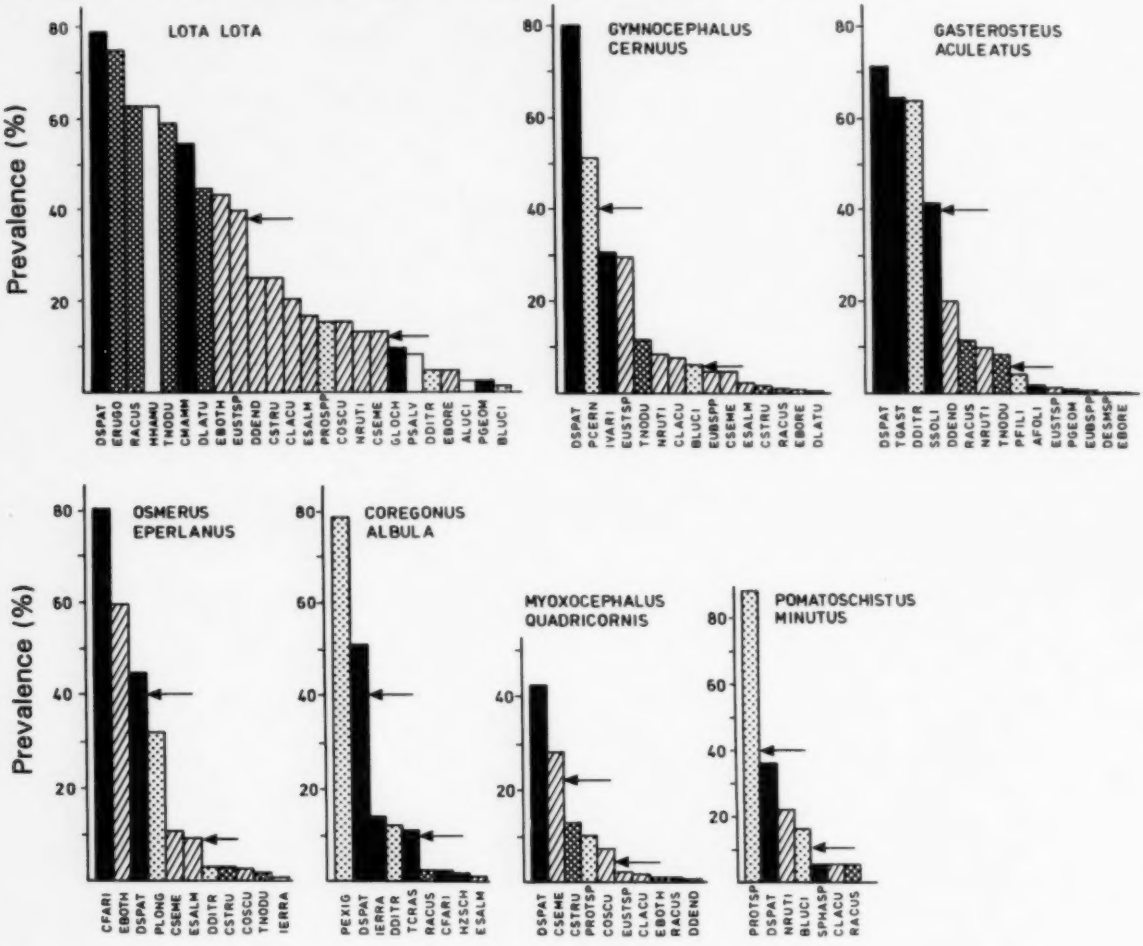

LOTA LOTA
GYMNOCEPHALUS CERNUUS
GASTEROSTEUS ACULEATUS
OSMERUS EPERLANUS
COREGONUS ALBULA
MYOXOCEPHALUS QUADRICORNIS
POMATOSCHISTUS MINUTUS
Prevalence (%)
80
60
40
20
DSPAT ERUGO RACUS MHAMU TNODU CMAMM DLATU EBOTH EUSTSP DDEND CSTRU CLACU ESALM PROSPP COSCU NRUTI CSEME GLOCH PSALV DDITR EBORE ALUCI PGEOM BLUCI
DSPAT PCERN IVARI EUSTSP TNODU NRUTI CLACU BLUCI EUBSPP CSEME ESALM CSTRU RACUS EBORE DLATU
DSPAT TGAST DDITR SSOLI DDEND RACUS NRUTI TNODU PFIL AFOL EUSTSP PGEOM EUBSPP DESMSP EBORE
CFARI EBOTH DSPAT PLONG CSEME ESALM DDITR CSTRU COSCU TNODU IERRA
PEXIG DSPAT IERRA DDITR TCRAS RACUS CFARI HZSCH ESALM
DSPAT CSEME CSTRU PROTSP COSCU EUSTSP CLACU EBOTH RACUS DDEND
PROTSP DSPAT NRUTI BLUCI SPHASP CLACU RACUS

Table 5. Fish studied from the Bothnian Bay, Baltic Sea, during 1978.

	No. of fish examined	No. of species found	Mean no. of species	No. of parasites/ fish	Max.	Diversity, exp. *H'*	Percentage similarity	Jaccard's similarity
Lota lota	76	23	6.3	61.9	520	3.5	0.28	0.32
Gasterosteus aculeatus	93	15	3.0	14.3	89	2.4	0.41	0.35
Gymnocephalus cernuus	106	15	2.5	14.5	153	2.1	0.35	0.30
Osmerus eperlanus	122	11	2.6	26.9	234	1.8	0.68	0.46
Myoxocephalus quadricornis	73	10	0.9	5.8	55	0.8	0.07	0.05
Coregonus albula	84	9	1.8	15.7	100	1.5	0.41	0.34
Pomatoschistus minutus	10	7	2.1	27.5	67	nc	nc	nc

Note: The scales for similarity indices are 0.00–1.00. nc, not counted.

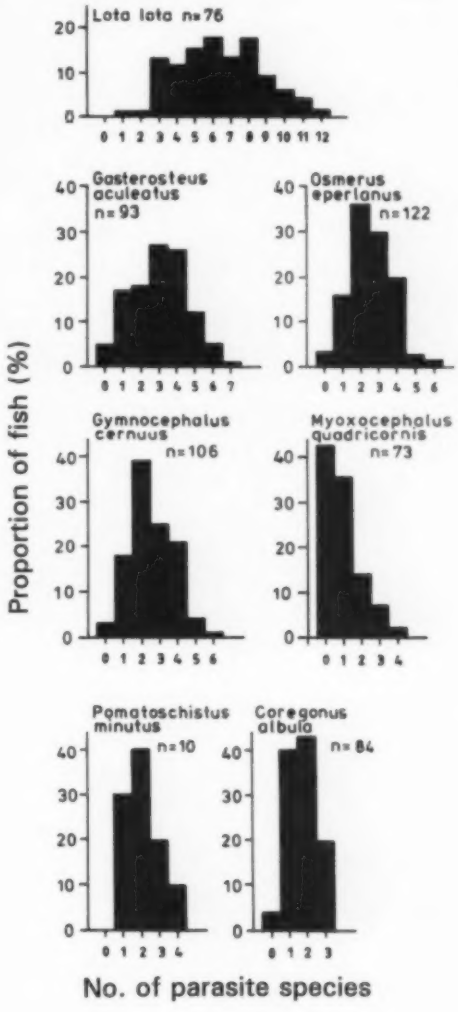
Lota lota also has the greatest parasite species richness (24), since it is exposed to many more parasite species via its prey fishes than are the prey fishes themselves. In addition to two important species of the prey fishes, many rare and secondary species are able to re-establish and even accumulate in *L. lota*. A total of 15 parasite species was recorded from both *Gymnocephalus cernuus* and *Gasterosteus aculeatus*, although the diet, as seen in Fig. 2, is broader in *G. cernuus*. However, the greatest proportion of food items in *G. aculeatus* is planktonic crustaceans, which are intermediate hosts for cestodes, and have not been identified to the same level as other food items. The low number of *Pomatoschistus minutus* studied may have accounted, to some extent, for the low number of parasite species found, because rarer species tend to be recovered only when larger numbers of fish are studied (Gregory and Blackburn 1991).

Lota lota shares 13 species with *G. cernuus* and 10 each with *G. aculeatus* and *M. quadricornis*. Because all fish species studied belong to different families, we cannot determine if transmission rates are higher between related species as shown by Leong and Holmes (1981). However, when the shared parasite species of two prey fishes, *C. albula* and *O. eperlanus*, which both belong to the suborder Salmonoidei, are compared, only three species are found in common, less than between *L. lota* and any prey fish species. *Pomatoschistus minutus* and *G. cernuus*, which both belong to the order Perciformes, share five species, the same number as *P. minutus* and *L. lota*. Further studies are therefore needed to investigate transmission of parasite species in fish hosts within the same family.

Of the 32 helminth species with complex life cycles, 21 species are shared between *L. lota* and some of the prey fishes. Most of these are transmitted via the food-web to *L. lota*. This is not the case for *Diplostomum spathaceum*, a generalist present in all fish species, and the more specific *Ichthyocotylurus* spp., which penetrates the fish directly. The remaining 11 helminths are specialists in some of the prey fish species or *L. lota*.

Of the four groups of parasites that are capable of being transmitted to *L. lota*, the first is composed of species that accumulate in *L. lota* through prey fishes. Of these, the cestodes *Triaenophorus nodulosus*, *Diphyllobothrium latum*, and *Eubothrium rugosum*, and the nematode *Raphidascaris acus* are important species of *L. lota*. In addition to *G. cernuus*, *G. aculeatus*, *O. eperlanus*, and *L. lota*, *Perca fluviatilis*, and *Pungitius pungitius* are also possible sources of *T. nodulosus* plerocercoids in the Bothnian Bay (Valtonen et al. 1989). It has been documented experimentally that plerocercoids of *D. latum* have the ability to re-establish in a new fish host when their host is eaten (Halvorsen and Wissler 1973; von Bonsdorff and Bylund 1982). Bylund (1969) and Halvorsen and Wissler (1973) have also shown that *D. dendriticum* can re-establish to some extent, too, but this capacity is poorly developed in *D. ditremum*. These results are supported by the present study, which shows that, while *D. dendriticum* is common in *L. lota*, *D. ditremum* was found only once in the stomach, even though it occurs in three prey-fish species and is even an important species of *G. aculeatus*. Because of the fact that

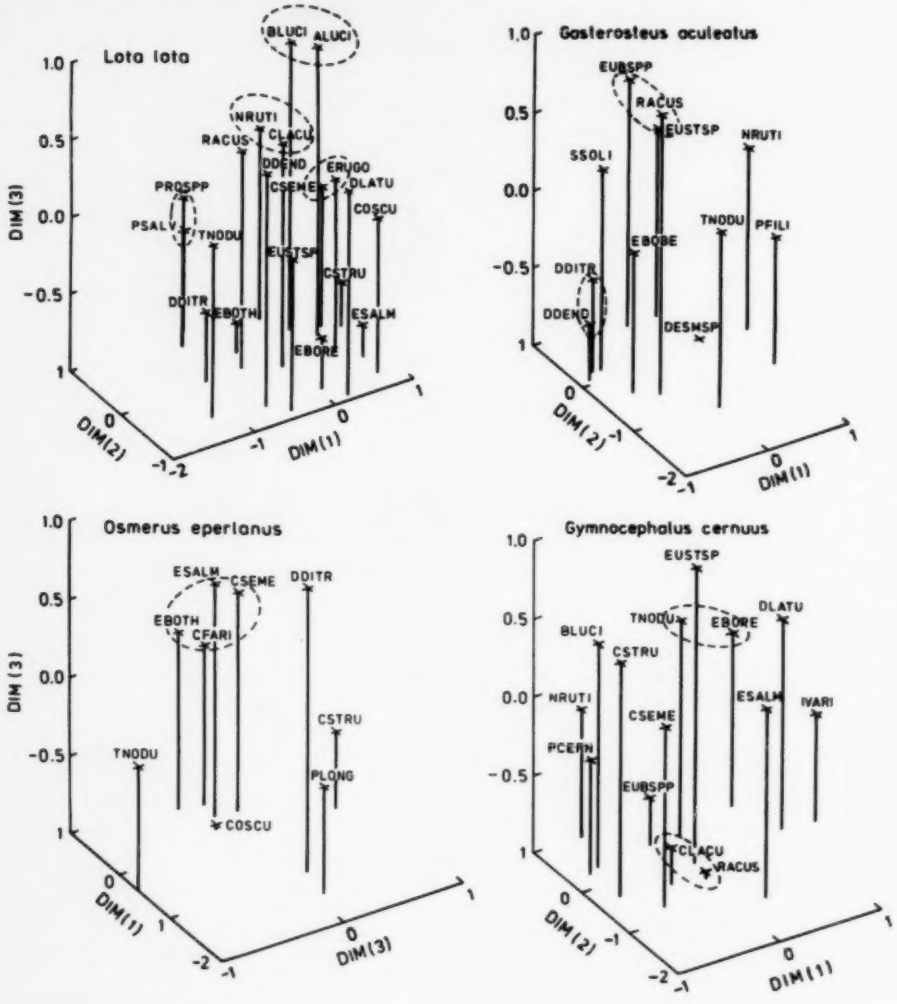
Fig. 6. Frequency distribution of the numbers of parasite species in seven fish species in the Bothnian Bay, Baltic Sea. *n*, sample size.



planktonic copepods are not eaten by adult *L. lota*, the important species *Eubothrium rugosum* must have been acquired from *G. cernuus* and *G. aculeatus*, where its juvenile stages were found. It is also possible that *E. rugosum* may be transmitted to *L. lota* via infected copepods present in the stomach contents of prey fishes.

The second group is formed by parasites that survive in *L. lota* for a limited period but cannot develop or establish. These include adult proteocephalids, plerocercoids of *D. ditremum*, and the adult trematode *Bunodera luciopercae*, which is also common in *Perca fluviatilis* in the study area (Rahkonen et al. 1984). The common, abundant, and host-specific adult proteocephalids found in five prey fish species occur as important species in *G. cernuus*, *C. albula*, and *M. quadricornis*, but only juveniles were

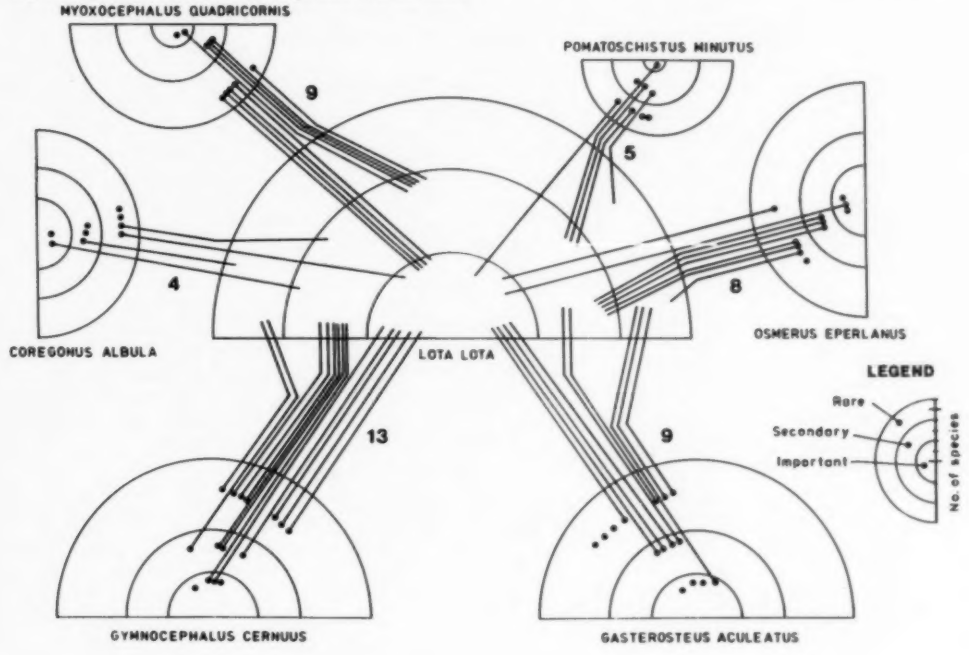
Fig. 7. Multidimensional scaling based on the Spearman correlation matrix of parasites for three fish species from the Bothnian Bay, Baltic Sea. Associated parasite species are indicated by dotted lines. DIM(1), DIM(2), and DIM(3) are the three dimensions. Parasite names are abbreviated as follows: first letter of the generic name together with first four letters of the species name. If the species is given as sp. or spp. then the first four to three letters of the generic name, respectively, have been used.



found in *L. lota* (see also Rintamäki and Valtonen 1988; Valtonen and Rintamäki 1989). Whether these juveniles are able to re-establish in *L. lota* from prey fish or are acquired from infected first intermediate hosts (copepods) present in the stomach contents of the prey is not known. However, it was apparent that none of the juvenile proteocephalids were able to survive very long in *L. lota* and none of the numerous mature adult proteocephalids in the prey fishes could re-establish in it. Andersen and Valtonen (1990) also noted that the exchange of adult cestodes between 19 infected fish species of the 31 fish species studied in the Bothnian Bay was rare, as was their co-existence in individual fish. Holmes (1979) also pointed out that adult *Eubothrium salvelini* (Schrank, 1790) in its required final host in Cold Lake, Alberta, was not transmitted to other fish species.

In the third group are parasites shared between *L. lota* and some of the prey fishes whose prevalences indicate no prominent accumulation or sometimes, even a decrease, in *L. lota* as compared with the prey fishes. This group includes, in addition to *Diphyllobothrium dendriticum*, both larval and adult nematodes and acanthocephalans. The post-cyclic transmission (see Nickol 1985) of adult nematodes between fish has been demonstrated by Moravec

Fig. 8. Patterns of possible transmission routes of parasites from the six prey fish species to *Lota lota* in the Bothnian Bay, Baltic Sea. The total numbers of shared species are also given. For the names of important, secondary, and rare species see the text.



(1969a), who documented the transfer of camallanids from small forage fishes to larger predatory species. In the present study, three prey fish species are found to have adult *Camallanus lacustris* as a rare or secondary species, although perch is the required final host of this species (see Holmes 1979), and may be the source of this nematode for predators (E.T. Valtonen, unpublished data; Moravec 1969*b*; Skorping 1980). The larval nematodes *Eustrongylides* sp. and *Contracaecum osculatum* (Rudolphi, 1802) can re-establish in *L. lota* from several prey fishes and *Raphidascaris acus*, which matures in *L. lota* and in *Esox lucius* (Moravec 1970*a*, 1970*b*; Fagerholm 1982), is acquired from five prey fishes. *Eustrongylides* sp. appeared healthy in *G. cernuus* but in other fishes, including *L. lota*, they were in poor condition, moribund, or dead. Post-cyclic transmission of adult acanthocephalans also appears to be common between *L. lota* and its prey fishes in the Bothnian Bay. All seven acanthocephalan species were shared between *L. lota* and three to seven prey fishes. Furthermore, juveniles encapsulated on the inner organs of fishes serving as paratenic hosts, as is the case for two *Corynosoma* species (Margolis 1958; Valtonen 1983*b*), may be able to re-establish in the tissues of *L. lota*. Most *Corynosoma* spp. mature in seals (Valtonen and Helle 1988). Evidence for the post-cyclic transmission of acanthocephalans has been shown experimentally in studies on the life history of *Neoechinorhynchus rutili*, other neoechinorhynchids, and *Echinorhynchus salmonis* (Hnath 1969; Uglem and Beck 1972; Lassiere and Crompton 1988). The intermediate hosts of acanthocephalans, ostracods, *Monoporeia affinis*, *Gammarus* spp., and *Mysis relicta* were found in the diet of *L. lota* (Valtonen and Crompton 1990). It appears then that acanthocephalan transmission to *L. lota* may also result from the ingestion of infective cystacanths present in intermediate hosts. However, cystacanths have very low prevalences in arthropods of the Bothnian Bay (Valtonen and Crompton 1990) and thus the post-cyclic transmission from prey fishes is suggested to be the most important way in which infections in *L. lota* are established, even if cystacanths are known to alter the behaviour of their intermediate hosts to increase the chances of transmission to the final hosts (Holmes and Zohar 1990). The fact that acanthocephalan prevalences in *L. lota* never exceeded those found in prey fish species is further evidence that intermediate hosts are rarely a direct source of infection to *L. lota*.

The fourth group includes specific parasites that are never shared between *L. lota* and its prey. These include plerocercoids of *Triaenophorus crassus* and *Schistocephalus solidus* (O.F. Müller, 1776), and the adult nematode *Cystidicola farionis*, which matures in the swimbladder of *O. eperlanus* but also occurs in *C. albula* and *C. lavaretus* (s.l.) of the Bothnian Bay (Valtonen and Valtonen 1978). In the case of *C. farionis*, this may result from the intrinsic nature of its specificity or because its migration from the esophagus to the swimbladder is prevented in *L. lota*, a physoclistic fish (Black and Lankester 1981). The source of the nematodes *Pseudocapillaria salvelini* and *Haplonema hamulatum*, found only in *L. lota*, is not known,

but they may have been transmitted as adult or larval stages from salmonids and *G. cernuus*, respectively (see Fagerholm 1982). Both of these species mature in *L. lota*. The two *Ichthyocotylurus* species in *O. eperlanus*, *C. albula*, and *G. cernuus*, and *Desmidocercella* sp. in *G. aculeatus* are not shared with *L. lota*.

It has been shown that some of the core species of the lesser scaup duck (*Aythya affinis*) form two suites of helminth species in bird infracommunities, with each suite using different intermediate hosts (Bush and Holmes 1986). The parasites in the present fish infracommunities were not clearly organized into suites, as revealed by the three-dimensional scaling, except for the four species in *O. eperlanus*, which all use the amphipods *Monoporeia affinis* or *Mysis relicta* as intermediate hosts (Valtonen and Crompton 1990). These arthropods are found in similar environments in the Bothnian Bay. Two cestode plerocercoids that use copepods as intermediate hosts were also grouped together in *G. aculeatus* (see also Andersen and Valtonen 1992). However, in both cases the parasite species in the suite in the prey fish were not found in *L. lota*. This demonstrates the importance of evolutionary processes such as host specificity for the structuring of the parasite community.

Our results are comparable to some extent with the large study by Leong and Holmes (1981) of nine freshwater fish species from Cold Lake, Alberta. The present study area is more like a cold oligotrophic lake than a sea when its salinity (3‰) and fish fauna (Andreasson and Petersson 1982) are taken into consideration. Furthermore, the balance between allogenic and autogenic parasite species is in favour of autogenic species, which is typical for oligotrophic water bodies (see Wisniewski 1958; Esch 1971). Leong and Holmes (1981) found 37 parasite species in Cold Lake versus the 39 that were encounted in the present study. They studied the same parasite groups as in the present case, although they included also monogeneans, whereas we did not. The greatest number of species found in Cold Lake, 16 in the lake whitefish, *Coregonus clupeaformis*, was less than in the present results, and the least number was three in the walleye, *Stizostedion vitreum*. In Cold Lake, the mean number of parasite species per infected fish varied from 4.6 in lake whitefish to 1.8 in the white sucker, *Catostomus commersoni*. These values are roughly in line with those found in the present study, where the range was 1.8–6.3 per fish. An interesting feature is the low species richness of *L. lota* in Cold Lake. Only six species were found, although the mean number of parasites per fish was almost four times greater than in *L. lota* from the Bothnian Bay. The small sample size of *L. lota* in Cold Lake may, however, account for the low total number of species found (Gregory and Blackburn 1991). Nevertheless, the results from the Bothnian Bay confirm the findings of Kennedy et al. (1986) and Janovy et al. (1992), that parasite communities of ectothermic animals, such as fish, both predators and prey fishes, are depauperate when compared with birds and mammals, although the predator examined in this study had higher parasite species richness and more variable infracommunities than its prey. Although parasite specificity was fundamental to determining if the parasite species available to the predator through its diet could establish or not, the increased variety of food objects increased the richness of parasite communities of the predator fish.

Acknowledgements

This research was financed by the Research Councils for the Natural and Environmental Sciences of the Academy of Finland. We express our sincere thanks to Dr. A. Shostak, Prof. J.C. Holmes, and Dr. J.R. Arthur for valuable comments on the paper; Drs. G. Bylund, H.P. Fagerholm, and J. Särkkä for discussions; and Dr. Jouni Taskinen for reading and commenting on the paper.

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Parasites of capelin (*Mallotus villosus*) in the St. Lawrence estuary and gulf

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Abstract: A survey of the parasites of capelin (*Mallotus villosus*) caught on the spawning grounds at five localities in the estuary of the St. Lawrence River and the Gulf of St. Lawrence, Canada, was conducted in 1994. From examination of 125 fish, a total of 21 parasite taxa was identified (2 Protista, 3 Monogenea, 4 Digenea, 4 Cestoda, 7 Nematoda, and 1 Acanthocephala). Seven new host records are reported for this fish (*Trichodina* sp., *Brachyphallus crenatus*, *Bothrimonus sturionis*, *Ascarophis* sp., *Pseudoterranova decipiens* larva, Spirurida gen.sp. larva, and *Echinorhynchus laurentianus*). The parasite fauna of capelin is comparatively depauperate. It is characterized by the relative abundance of a few species showing a high level of host specificity (*Microsporidium* sp., *Trichodina* sp., *Gyrodactyloides andriaschewi*, *G. petruschewskii*, *Laminiscus gussevi*, and *Eubothrium parvum*) that mature on or in capelin, several ubiquitous adult digeneans (e.g., *B. crenatus*, *Derogenes varicus*, *Hemiurus levinseni*, and *Lecithaster gibbosus*), and a number of species that use capelin as intermediate or transport hosts (e.g., *Scolex pleuronectis* plerocercoid, *Anisakis simplex* larva, Contracaecinea gen.sp. larva, and *Hysterothylacium aduncum* larva).

Résumé : La faune parasitaire de capelans (*Mallotus villosus*) capturés à cinq sites de frai dans l'estuaire et le golfe du Saint-Laurent, au Canada, a été étudiée en 1994. Au total, 21 taxons de parasites ont été identifiés (2 protistes, 3 monogènes, 4 digènes, 4 cestodes, 7 nématodes et 1 acanthocéphale) après examen de 125 poissons. Parmi ceux-ci, sept taxons n'ont jamais été répertoriés auparavant chez ce poisson (*Trichodina* sp., *Brachyphallus crenatus*, *Bothrimonus sturionis*, *Ascarophis* sp., *Pseudoterranova decipiens* larvaire, Spirurida gen.sp. larvaire et *Echinorhynchus laurentianus*). La faune parasitaire du capelan est comparativement pauvre et est principalement caractérisée par l'abondance relative de quelques espèces démontrant un haut niveau de spécificité (*Microsporidium* sp., *Gyrodactyloides andriaschewi*, *G. petruschewskii*, *Laminiscus gussevi* et *Eubothrium parvum*) se développant à maturité sur ou dans le capelan, par plusieurs digènes adultes généralistes (p. ex., *B. crenatus*, *Derogenes varicus*, *Hemiurus levinseni* et *Lecithaster gibbosus*) et par plusieurs espèces qui utilisent le capelan comme hôte intermédiaire ou de transport (*Scolex pleuronectis* plérocercoïde, *Anisakis simplex* larvaire, Contracaecinea gen.sp. larvaire et *Hysterothylacium aduncum* larvaire).

The capelin (*Mallotus villosus*) is a small cold-water pelagic fish of the family Osmeridae (smelts) of circumpolar distribution occurring in the northern parts of the North Atlantic and North Pacific oceans (Scott and Scott 1988). Capelin are a key component in the aquatic food web in the northwest Atlantic Ocean, where they constitute a major food item for many species of marine and anadromous fishes, seabirds, seals, and cetaceans (see Scott and Scott 1988). With declining fishery resources in the Gulf of St. Lawrence, the capelin is also becoming increasingly important as a commercial species (Anonymous 1994). A roe fishery for the Japanese market in the northern Gulf (NAFO Divisions 4RS) and a smaller fishery in the southern Gulf (Division 4T) for animal consumption accounted for a total catch of more than 10 000 t in 1993 (Anonymous 1994).

Despite the ecological and economic importance of the capelin, its parasite fauna has been little studied. The only major survey conducted is that of Pálsson (1982), who examined fish taken from the Atlantic Ocean off Canada and Iceland, and from the Barents Sea. This extensive study identified 15 helminth taxa (3 Monogenea, 3 Digenea, 5 Cestoda, 3 Nematoda, and 1 Acanthocephala) from examination of more than 3500 capelin (see Pálsson 1982, 1986; Pálsson and Beverley-Burton 1984). Small numbers of capelin have also been examined as part of general surveys of parasites of fishes of the western Bering Sea (Zhukov 1960, 1963) and the Barents Sea (Polyansky 1955). Other scattered records of parasites from this host occur in the taxonomic literature (Nybelin 1922; Hansen 1923; Isaichikov 1928; Marchant and Schiffman 1946; Bykhovsky 1947; Bykhovsky and Polyansky 1953; Margolis and Ching 1965; Tsimbalyuk and Semeshko 1971; Fröiland 1974; Andersen 1979; Vávra and Undeen 1979; Love and Moser 1983; Pálsson and Beverley-Burton 1983) and in studies on capelin life history (Pigeon and Vallée 1937; Templeman 1948, 1968; Templeman et al. 1957; Winters 1970; Kennedy 1979). Together, these various studies report a total of only 19 parasite taxa.

The purpose of the present communication is to provide basic survey information on the parasites of capelin in the Gulf of St. Lawrence. We also examine the peculiarities of the parasite fauna of this fish, including the likely

Received March 31, 1995. Accepted May 17, 1995.
J12848

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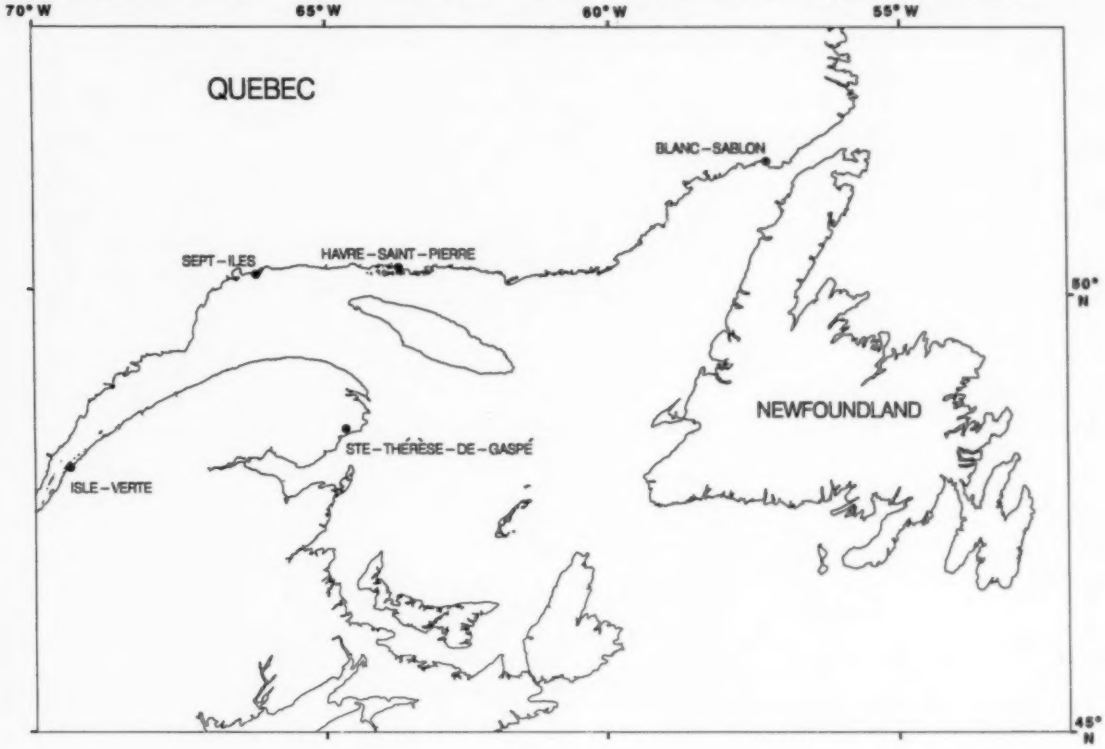
Fig. 1. Map of the estuary of the St. Lawrence River and the Gulf of St. Lawrence showing collection localities.



pathways by which capelin become infected with the various species, and provide a brief summary of the parasites reported from capelin throughout its geographic range. The use of parasites as potential "biological tags" for capelin in the St. Lawrence estuary and gulf will be examined in a subsequent paper.

Materials and methods

A total of 125 male capelin collected from the five localities within the estuary of the St. Lawrence River and the Gulf of St. Lawrence designated on Fig. 1 were examined during the course of this study. Fish were collected by trap-net, dip-net, or purse seine on the spawning grounds during May–July 1994.

Fish were individually bagged and deep frozen immediately after capture for later laboratory examination. Host data are summarized below with the following information presented for each collection: locality (Fig. 1), dates of collection (d, mo, yr), number of fish examined, and their total length (mm) and weight (g) given as the mean ± SD followed by the range.

1. Isle-Verte, 13-05-94 and 01-06-94, n = 25, 155.5 ± 5.3 mm (146–163), 20.6 ± 2.2 g (16.2–24.3).
2. Sept-Îles, 03-06-94 and 08-06-94, n = 25, 154.5 ± 4.6 mm (145–161), 21.1 ± 2.1 g (16.9–24.8).
3. Havre-Saint-Pierre, 08-06-94 and 14-06-94, n = 25, 155.5 ± 4.5 mm (147-164), 20.8 ± 1.9 g (16.7–24.7).
4. Blanc-Sablon, 30-06-94 and 05-07-94, n = 25, 157.0 ± 6.1 mm (144–168), 23.5 ± 3.1 g (17.3–29.2).
5. Sainte-Thérèse-de-Gaspé, 16-06-94, n = 25, 149.7 ± 4.3 mm (141–158), 19.6 ± 2.0 g (16.2–24.3).

Complete examinations for protistan and metazoan parasites were performed using standard parasitological methods. In the laboratory, fish were thawed, measured, and weighed. The external surface including fins was examined for ectoparasites using a stereomicroscope. Gills were removed and arches examined individually. The buccal cavity, opercula, and eyes were examined separately. The internal organs (heart, liver, spleen, gallbladder, digestive tract, gonads, swim bladder, kidney, and urinary bladder) were inspected for parasites free or encapsulated on their outer surfaces, separated, and then individually examined. The stomach, pyloric caeca, and intestine were separated, opened longitudinally, and their contents rinsed into beakers where they were mixed with sodium bicarbonate and settled to recover endoparasitic helminths. The liver, spleen, kidney, and heart were compressed between glass plates and examined for parasites. Squash preparations made from liver, spleen, kidney, testes, intestine, muscle, and brain tissue, and scrapings from the urinary and gallbladders were examined using a compound microscope equipped with interference-contrast optics at a magnification of 400×. Preparations not found to harbour protistans after a 5-min examination were considered uninfected. The body musculature was removed from the vertebral column, the

Table 1. Summary of parasites of capelin (*Mallotus villosus*) sampled in the estuary of the St. Lawrence River and the

Parasite	Location[a]	Isle-Verte (n = 25)		Sept-Îles (n = 25)	
		P (%)[b]	Intensity	P (%)	Intensity
Protista					
Microsporidium sp.	mes	16.0	—	36.0	—
Trichodina sp.	Gills	0.0	—	24.0	—
Monogenea					
Gyrodactyloides andriaschewi	Gills	88.0	22.5±27.4 (2–128)	100.0	48.0±56.7 (4–206)
G. petruschewskii	Gills	76.0	12.7±34.2 (1–152)	92.0	16.7±38.8 (1–191)
Laminiscus gussevi	Gills	96.0	6.2±5.9 (1–29)	100.0	10.5±8.1 (2–37)
Total		100.0	36.8±56.7 (1–296)	100.0	81.7±109.0 (14–549)
Digenea					
Brachyphallus crenatus	sto	—	—	—	—
Derogenes varicus	sto	4.0	1.0±0.0 (1)	12.0	1.0±0.0 (1)
Hemiurus levinseni	sto	—	—	—	—
Lecithaster gibbosus	pc, int	4.0	1.0±0.0 (1)	8.0	1.5±0.7 (1–2)
Cestoda					
Bothrimonus sturionis	int	—	—	4.0	3.0±0.0 (3)
Eubothrium parvum	pc	8.0	1.0±0.0 (1)	—	—
Pseudophyllidea gen.sp. (p)[c]	sw, pc, int	—	—	—	—
Scolex pleuronectis (p)	pc, int	80.0	5792.0±4866.1 (170–17450)	92.0	14653.3±15212.1 (10–60100)
Nematoda					
Anisakis simplex (l)[c]	bc, mes, sw, mus	28.0	1.0±0.0 (1)	12.0	1.7±1.2 (1–3)
Ascarophis sp.	sto	—	—	—	—
Contracaecinea gen.sp. (l)	bc, mes	80.0	2.6±1.3 (1–6)	72.0	2.7±1.7 (1–7)
Hysterothylacium aduncum (l)	bc, mes, sw	—	—	4.0	1.0±0.0 (1)
Pseudoterranova decipiens (l)	mus	—	—	—	—
Spirurida gen.sp. (l)	sw	—	—	—	—
Nematoda gen.sp. (l)	iw	4.0	1.0±0.0 (1)	4.0	1.0±0.0 (1)
Acanthocephala					
Echinorhynchus laurentianus	int	—	—	4.0	1.0±0.0 (1)

[a]bc, body cavity; int, intestine; iw, intestinal wall; mes, mesenteries; mus, musculature; pc, pyloric caeca; sto, stomach; sw, stomach wall.
[b]P (%), prevalence (percent infected). Intensities are given as the mean ± SD, followed by the range in parentheses.
[c]p, plerocercoid; l, larva.

skin removed from the fillets, and the fillets pressed between glass plates and inspected for helminths and protistan cysts using a stereomicroscope. The above techniques are effective for the recovery of all parasites except for external protistans, which are typically destroyed by freezing, and hematozoans.

All parasites were sorted by taxon, cleaned, and counted for each organ (metazoans only). Microsporidians were fixed in 10% buffered formalin, nematodes in 70% ethanol with 10% glycerin, and digeneans, cestodes, and acanthocephalans in alcohol–formalin–acetic acid (AFA) for later identification to lowest possible taxon. For identification of species, monogeneans were examined unfixed as temporary wetmounts using a compound microscope, following which, representative specimens were fixed in AFA. Because of the extremely high number of plerocercoids of *Scolex pleuronectis* O.F. Müller, 1788, in some fish (up to 145 500), numbers of this parasite in heavily infected capelin were estimated by dilution of the sample to a known volume and subsampling. As necessary, digeneans, cestodes, and acanthocephalans were stained with Schneider's acetocarmine, dehydrated through a graded alcohol series, cleared in methyl salicylate, and mounted in Permount. Nematodes were cleared by evaporation in glycerin in 70% ethanol and examined as temporary mounts. All unmounted metazoan parasites were transferred to 10% glycerin in 70% ethanol for long-term storage.

Voucher specimens for all taxa were deposited in the collections of the Canadian Museum of Nature as collection Nos. CMNP1995-0090 to CMNP1995-0124 and in the parasitological collection of the Maurice Lamontagne Institute.

Parasite data are presented as prevalence (% infected) and intensity of infection (number of parasites per infected fish), expressed as the mean ± the standard deviation, followed by the range as recommended by Margolis et al. (1982). Parasites are considered "common" when their prevalences exceed 25% in any collection, "uncommon" when between 5 and 25%, and "rare" when less than 5%.

Gulf of St. Lawrence.

Havre-Saint-Pierre (*n* = 25)		Blanc-Sablon (*n* = 25)		Sainte-Thérèse-de-Gaspé (*n* = 25)	
P (%)	Intensity	*P* (%)	Intensity	*P* (%)	Intensity
16.0	—	16.0	—	24.0	—
16.0	—	40.0	—	40.0	—
100.0	82.2±282.8 (2–1434)	100.0	155.0±358.9 (3–1773)	100.0	100.0±149.6 (15–795)
92.0	16.3±20.6 (2–85)	96.0	40.6±136.2 (1–677)	100.0	20.9±24.1 (2–116)
92.0	5.6±7.6 (1–38)	80.0	2.4±1.5 (1–6)	72.0	2.9±2.7 (1–9)
100.0	109.7±313.7 (5–1601)	100.0	205.0±505.2 (5–2543)	100.0	129.2±172.6 (29–924)
—	—	—	—	56.0	2.0±1.2 (1–4)
—	—	—	—	—	—
—	—	—	—	4.0	1.0±0.0 (1)
—	—	4.0	5.0±0.0 (5)	—	—
—	—	—	—	—	—
4.0	1.0±0.0 (1)	32.0	3.9±3.4 (1–9)	16.0	1.0±0.0 (1)
—	—	4.0	1.0±0.0 (1)	—	—
84.0	10622.9±15898.3 (1–71500)	92.0	16983.0±31298.3 (4–145500)	96.0	19352.1±17914.2 (710–73600)
24.0	1.2±0.4 (1–2)	12.0	1.0±0.0 (1)	12.0	1.3±0.6 (1–2)
—	—	—	—	4.0	1.0±0.0 (1)
72.0	2.0±1.0 (1–5)	92.0	2.3±1.7 (1–8)	60.0	1.3±0.6 (1–3)
4.0	1.0±0.0 (1)	4.0	1.0±0.0 (1)	8.0	1.0±0.0 (1)
—	—	—	—	4.0	1.0±0.0 (1)
—	—	4.0	1.0±0.0 (1)	—	—
8.0	1.0±0.0 (1)	4.0	1.0±0.0 (1)	4.0	1.0±0.0 (1)
4.0	2.0±0.0 (2)	—	—	—	—

Results

Among the 21 parasite taxa identified from capelin during this study are 2 protistans, 3 monogeneans, 4 digeneans, 4 cestodes, 7 nematodes, and 1 acanthocephalan (Table 1). Prevalences for *Trichodina* sp. given in Table 1 are undoubtedly lower than actual values, because of the probable destruction of most specimens of this ectoparasitic ciliate during host freezing.

A brief explanation of problems encountered during the identification of several of the parasite species infecting capelin is required. Microsporidians infecting the mesenteries of capelin were assigned to the collective group *Microsporidium* following Canning and Lom (1986). Their identification must await more detailed studies employing transmission electron microscopy. Specific identification of *Trichodina* sp. requires collection of fresh material for silver impregnation.

A few helminths could not be assigned to species because of their larval state. These include plerocercoids of Pseudophyllidea gen.sp. and *Scolex pleuronectis* (a collective grouping for tetraphyllidean plerocercoids), and nematode larvae belonging to Contracaecinea gen.sp., Spirurida gen.sp., and Nematoda gen.sp. The latter category includes extremely tiny (length approximately 300 μm) larvae seen occasionally in squashes of intestinal tissue being examined for protistan infections. Only immature *Echinorhynchus laurentianus* Ronald, 1957 and *Ascarophis* sp. were found. Although an attempt was made to identify all Monogenea, this was not possible, because approximately 10% of all specimens were damaged during their removal from the gill filaments. Table 1 thus presents data for all specimens identified to species, as well as total counts for all monogeneans combined.

Included in our findings are a number of parasites not previously reported from capelin. These include the ciliate *Trichodina* sp.; the digenean *Brachyphallus crenatus* (Rudolphi, 1802); the cestode *Bothrimonus sturionis* Duvernoy, 1842; the acanthocephalan *Echinorhynchus laurentianus*; and the nematodes *Ascarophis* sp.;

Table 2. Summary of parasites reported from capelin (*Mallotus villosus*).[a]

Parasite	Location[b]	Locality[c]	Frequency[d]	Reference[e]
Protista				
Ceratomyxa sp.	ub	BAR	Uncommon	16
Microsporidium sp.				
Includes *Glugea* sp. auctorum; *Nosema* sp. of Polyansky, 1955	bc, gon, liv, mes, mus	BAR, NWA	Common	9, 16, 17, 18, 21, 25
Trichodina sp.	Gills	NWA	Common	25
Monogenea				
Gyrodactyloides andriaschewi Bykhovsky and Polyansky, 1953	Gills	BAR, NEA, NWA, WBE, NWP	Common	3, 12, 13, 14, 15, 16, 23, 25
G. petruschewskii Bykhovsky, 1947	Gills	BAR, NEA, NWA, WBE, NWP	Common	2, 3, 12, 13, 14, 15, 16, 23, 25
Laminiscus gussevi (Bykhovsky and Polyansky, 1953)				
Syn.: *Gyrodactyloides gussevi* Bykhovsky and Polyansky, 1953	Gills	BAR, NEA, NWA, WBE	Common	3, 12, 13, 14, 15, 16, 23, 25
Digenea				
Brachyphallus crenatus (Rudolphi, 1802)	sto	NWA	Common	25
Derogenes varicus (O.F. Müller, 1784)	sto	NWA	Uncommon	12, 13, 15, 25
Hemiurus levinseni Odhner, 1905	sto	NWA	Uncommon	12, 13, 15, 25
Lecithaster confusus Odhner, 1905	int	BAR	Uncommon	6
L. gibbosus (Rudolphi, 1802)	int, pc	NEA, NWA, WBE	Common	12, 13, 15, 24, 25
Pronoprymna petrowi (Layman, 1930)				
Syn.: *Pentagramma petrowi* (Layman, 1930)	int or pc	NEP	Common	10
Cestoda				
Bothrimonus sturionis Duvernoy, 1842	int	NWA	Rare	25
Diphyllobothrium sp. plerocercoid	int, sto, sw	NEA, NWA, NWP	Common	12, 15, 20
Eubothrium parvum Nybelin, 1922	int	BAR, NEA, NWA	Uncommon	1, 7, 11, 12, 13, 15, 17, 18, 25
Pseudophyllidea gen.sp. plerocercoid	int, pc, sto, sw	NWA	Rare	12, 15, 25
Scolex pleuronectis O.F. Müller, 1788 plerocercoid				
Syn.: *S. polymorphus* Rudolphi, 1819				
Includes Tetraphyllidea larva auctorum	int, pc, sto(?)	BAR, NWA, WBE	Common	12, 15, 16, 24, 25
Nematoda				
Anisakis simplex (Rudolphi, 1809) larva				
Includes *Anisakis* sp. auctorum	bc, mes, mus, sw	BAR, NEA, NWA, NEP, NWP, WBE	Common	8, 12, 13, 15, 16, 19, 24, 25
Ascarophis sp.	sto	NWA	Rare	25
Contracaecinea gen.spp. larva				
Includes *Contracaecum clavatum* larva of Templeman, 1948; *Contracaecum* sp. larva auctorum	bc, gon, liv, mes, mus, sbw	BAR, NEA, NWA	Common	12, 13, 15, 17, 18, 19, 22, 25

Table 2 (*concluded*).

Parasite	Location[b]	Locality[c]	Frequency[d]	Reference[e]
Hysterothylacium aduncum (Rudolphi, 1802) larva Syn.: *Contracaecum aduncum* (Rudolphi, 1802); *Thynnascaris aduncum* (Rudolphi, 1802) Includes *Hysterothylacium* sp. of Pálsson and Beverley-Burton, 1984	bc, int, mes, sw	BAR, NEA, NWA, WBE	Common	12, 13, 15, 16, 24, 25
Pseudoterranova decipiens (Krabbe, 1878) larva	mus	NWA	Rare	25
Spirurida gen.sp. larva	sw	NWA	Rare	25
Acanthocephala				
Echinorhynchus gadi Zoega in O.F. Müller, 1776	int	NWA	Rare	12, 15
E. laurentianus Ronald, 1957	int	NWA	Rare	25
Copepoda				
Haemobaphes cyclopterina (Fabricius, 1780)	Gills	BAR	Rare	4, 5

[a]Does not include the reports of unidentified nematodes and digeneans from capelin by Pigeon and Vallée (1937).

[b]bc, body cavity; gon, gonad; int, intestine; liv, liver; mes, mesenteries; mus, musculature; pc, pyloric caeca; sbw, swimbladder wall; sto, stomach; sw, stomach wall; ub, urinary bladder.

[c]BAR, Barents Sea; NEA, Northeast Atlantic Ocean; NWA, Northwest Atlantic Ocean; NEP, Northeast Pacific Ocean; NWP, Northwest Pacific Ocean; WBE, Western Bering Sea.

[d]Common, uncommon, and rare are maximum reported prevalences of >25%, >5% to <25%, and <5%, respectively, in any one host collection.

[e]1, Andersen 1979; 2, Bykhovsky 1947; 3, Bykhovsky and Polyansky 1953; 4, Fröiland 1974; 5, Hansen 1923; 6, Isaichikov 1928; 7, Kennedy 1979; 8, Love and Moser 1983; 9, Marchant and Schiffman 1946; 10, Margolis and Ching 1965; 11, Nybelin 1922; 12, Pálsson 1982; 13, Pálsson 1986; 14, Pálsson and Beverley-Burton 1983; 15, Pálsson and Beverley-Burton 1984; 16, Polyansky 1955; 17, Templeman 1948; 18, Templeman 1968; 19, Templeman et al. 1957; 20, Tsimbalyuk and Semeshko 1971; 21, Vávra and Undeen 1979; 22, Winters 1970; 23, Zhukov 1960; 24, Zhukov 1963; 25, Arthur et al. (this paper).

Pseudoterranova decipiens (Krabbe, 1878) larva; and Spirurida gen.sp. larva.

Capelin occurring in the estuary of the St. Lawrence River and the Gulf of St. Lawrence are infected by a rather limited parasite fauna, only 21 taxa being encountered. Of these, only 29% (6 of 21 taxa) were common (prevalences greater than 25%) at more than one of the five localities. These include *Trichodina* sp.; *Gyrodactyloides andriaschewi* Bykhovsky and Polyansky, 1953; *G. petruschewskii* Bykhovsky, 1947; *Laminiscus gussevi* (Bykhovsky and Polyansky, 1953); *S. pleuronectis*; and Contracaecinea gen.sp. larva. Four species (19%), *Microsporidium* sp.; *Brachyphallus crenatus*; *Eubothrium parvum* Nybelin, 1922; and *Anisakis simplex* (Rudolphi, 1808) larva, were each common in only one collection.

An additional four taxa (19%) can be considered uncommon parasites, occurring with a maximum prevalence of 5–25% in any one collection and in much lower intensities of infection. These include *Derogenes varicus* (O.F. Müller, 1784); *Lecithaster gibbosus* (Rudolphi, 1802); *Hysterothylacium aduncum* (Rudolphi, 1802) larva; and Nematoda gen.sp. larva. The remaining six taxa (29%) were rare, occurring in only one or two of all fish examined (prevalences less than 5% in any single collection). These include *Hemiurus levinseni* Odhner, 1905; *Bothrimonus sturionis* Duvernoy, 1842; Pseudophyllidea gen.sp. plerocercoid; *Ascarophis* sp.; *P. decipiens* larva; Spirurida gen.sp. larva; and *E. laurentianus*.

Discussion

As shown by this, and by the previous studies of Pálsson (1982), Polyansky (1955), and Zhukov (1960, 1963), the parasite fauna of capelin is depauperate in comparison to that of many other marine fishes, only a total of 26 taxa having been reported from this fish throughout its entire geographic range (see Table 2). It is characterized by the relative abundance of a few species that are strictly or primarily parasites of capelin (*Microsporidium* sp., *Trichodina* sp., *Gyrodactyloides petruschewskii*, *G. andriaschewi*, *Laminiscus gussevi*, and *Eubothrium parvum*), a few digeneans (primarily Hemiuroidea) that mature in capelin and a wide variety of other marine fishes (*Brachyphallus crenatus*; *Derogenes varicus*; *Hemiurus levinseni*; *Lecithaster gibbosus*; *L. confusus* Odhner, 1905; and *Pronoprymna petrowi* (Layman, 1930)), and cestode plerocercoids and larval nematodes, likewise ubiquitous in marine fishes of northern latitudes, that use capelin as intermediate or transport hosts (*Diphyllobothrium* sp. plerocercoid, *Scolex pleuronectis* plerocercoid, *Anisakis simplex* larva, Contracaecinea gen.sp. larva, and *Hysterothylacium aduncum* larva).

Capelin are noteworthy in that they appear to lack myxosporean infections, which are typically common and often diverse in marine fishes. The only report of these parasites in capelin is that of Polyansky (1955), who reported plasmodia of a *Ceratomyxa* sp. in the urinary bladder of fish originating from the Barents Sea. Also notably absent are parasitic copepods, the only records from capelin being those of Hansen (1923) and Fröiland (1974), who each found a single specimen of *Haemobaphes cyclopterina* (Fabricius, 1780) on capelin collected in the Barents Sea and northeast Atlantic Ocean. Capelin are also characterized by their distinctive and often abundant monogenean fauna comprised of three species of gyrodactylids, *Gyrodactyloides andriaschewi*, *G. petruschewskii*, and *Laminiscus gussevi*.

The narrow range of heteroxenous parasites found in capelin is undoubtedly related to the capelin's relatively restricted diet. Capelin in the Gulf of St. Lawrence are known to feed heavily on planktonic crustaceans, with calanoid copepods and euphausiids being particularly important food items (Able et al. 1976). Other organisms eaten occasionally by capelin include amphipods, decapods, chaetognaths, and larval fishes (Pigeon and Vallée 1937; Able et al. 1976). Parasites transmitted to capelin via feeding on copepods include all four species of adult digeneans (*B. crenatus*, *D. varicus*, *H. levinseni*, and *L. gibbosus*), three cestodes (*S. pleuronectis*, *E. parvum*, and pseudophyllidean metacestodes), and the larvae of the nematodes *H. aduncum* and probably also the Contracaecinea (see Gibson and Bray 1986; Anderson 1992; Williams and Jones 1994). The frequent presence in capelin of the nematode *Anisakis simplex* larva, a species that uses the euphausiids *Thysanoessa raschii* and *Meganyctiphanes norvegica* as intermediate hosts in the Gulf of St. Lawrence (L. Measures, Maurice Lamontagne Institute, Mont-Joli, QC, personal communication), reflects the importance of these crustaceans in the diet. Similarly, our finding of the cestode *Bothrimonus sturionis* and the acanthocephalan *Echinorhynchus laurentianus* indicates that capelin occasionally ingest amphipods, the intermediate hosts for these parasites (see Sandeman and Burt 1972; Williams and Jones 1994). Two other parasites that were rare in capelin, the nematodes *Ascarophis* sp. and *Pseudoterranova decipiens* larva, also have life cycles associated with the benthos (see Anderson 1992).

In summary, the parasite fauna of capelin is comparatively depauperate. It consists principally of species that are either highly host specific (for the most part, ectoparasites having direct life cycles) and ubiquitous parasites that are transmitted via zooplankton. The latter include a small number of adult digeneans infecting the digestive tract and a number of immature forms (primarily cestodes and nematodes) that use capelin as intermediate or transport hosts.

Acknowledgements

We thank Jean-Denis Lambert for arranging host samples and Hélène Dionne for assistance with necropsies. Lena Measures kindly provided critical comments on the manuscript. This work was partly funded through the Programme fédéral de développement des pêches du Québec (PFDPQ).

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National Research Council Canada
Ottawa, Canada K1A 0R6

Conseil national de recherches Canada
Ottawa, Canada K1A 0R6

Postage paid at Ottawa
Publication mail
Registration No. 5431

Port payé à Ottawa
Poste-publication
Enregistrement n° 5431

Canadian Journal of Fisheries and Aquatic Sciences

Volume 52, Supplement 1, 1995

Journal canadien des sciences halieutiques et aquatiques

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Canada

Printed in Canada by / Imprimé au Canada par
University of Toronto Press Inc.
Typesetting by / Typographie par
Aubut & Nadeau Services Inc.

0706-652X(1995)52+1;1-R